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Contents of previous issues of the JOURNAL OF ANIMAL SCIENCE are indexed in the Agricultural Index which is available in most libraries and in the November issue of each complete volume of the JOURNAL.

EFFECTIVENESS OF SELECTION
A SYMPOSIUM PRESENTED AT THE 1949 MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

Introduction

W. A. CRAFT

Regional Swine Breeding Laboratory

I HAVE been requested to serve for Dean Rice since he could not be here. It is a pleasure to do something for him but we all regret his inability to be with us.

We all know that selection has been used by man and by nature for a long time. Changes in respect to conformation and performance have resulted in populations as the breeders have applied their skills in forming and improving breeds of livestock. Recently it has seemed that certain economically important characters in the established breeds are acting stubbornly toward the breeder's efforts to extend improvement.

It is only within the last few years that data from meat producing animals have become available for study with respect to selection pressure being applied and the response being experienced by experimental investigators. Dean Rice arranged also for a discussion of the contribution which may be made by small laboratory animals to aid in selection studies. Today as a result of his efforts, we have the privilege of listening to a discussion by some of the men who are involved in animal breeding investigations. I deem it a privilege to preside over this meeting.

In Laboratory Animals

ARTHUR B. CHAPMAN

University of Wisconsin[1]

THREE questions which may be appropriately raised and at least partially answered at this symposium on selection are:

1. What livestock selection problems may be aided in their solution by laboratory animal selection experiments?

[1] Paper No. 441 from the Department of Genetics, University of Wisconsin.

2. What features should be incorporated in the plans and analyses of laboratory animal selection experiments so that the results may be most readily transferred to other species as a guide in solving their selection problems?

3. What have laboratory animal experiments already contributed to our understanding of the application of genetic theory to selection?

The solutions to many of the problems in livestock selection and mating systems have been worked out theoretically in terms of the statistical consequences of the Mendelian mechanism. Very few experimental checks of these logical conclusions have been made and could well be made with laboratory animals to learn the extent to which observation fails to conform to expectation and, if possible, the reason for any non-conformance. There are also many problems in livestock selection which have not been solved in theoretical terms. Here again laboratory animals could serve a useful purpose in piloting the way.

Some of the more specific questions which these cheaper, more rapidly reproducing animals might be asked to answer are:

1. What methods of combining data from the pedigree, the progeny and the individual itself give the highest predictability of the animal's transmitting ability?

2. What changes occur in heritability from generation to generation in populations in which selection, combined with various mating systems, is practiced?

3. To what extent is information on the genetic sources of variation and performance in inbred lines indicative of their linecross and topcross combining ability?

4. What is the relative effectiveness of a long-time selection program for several characters when selection is practiced for all characters within a single group compared to selection with differential emphasis on the different characters in different groups which will eventually be intermated and combined into one group?

5. To what extent may variations in the general level of environment modify selection effectiveness?

6. How common are genotypic and phenotypic disharmonies and other correlated effects in selected populations?

7. What influence may selection, with and without inbreeding, have on homozygosity?

8. Do the schemes of dialleling and polyalleling have practical utility in increasing the accuracy of selection?

9. What is the relative effectiveness and efficiency of repeated cycles of inbreeding (to different levels of inbreeding) and linecrossing with

selection between lines and combinations of lines as compared to selection within lines (to different levels of inbreeding) followed by line-crossing and reinbreeding and as compared to selection within non-inbred stock followed by wide crosses?

These are a few of the problems which suggest themselves to all of us. Their very number and intricacy ought to urge all of us who are interested in animal breeding to do all we can with our limited facilities to attack these and similar problems in as many ways as possible in both laboratory and farm animal selection experiments.

What kind of data do we need from laboratory animal selection experiments to allow the results to have maximum transferability to other species?

In answering this we can take as our starting point the unity of nature in terms of the physical basis of inheritance and the potential measurability of the components of phenotypic variation. With this as a base, it would appear important to know (quantitatively, if possible) the following about laboratory animal selection experiments if they are to be of maximum value in helping to solve livestock selection problems:

1. The kind of selection practiced—the extent to which it was individual, pedigree and progeny selection.

2. The kind of mating system used—whether random mating, outbreeding, inbreeding, or assortive mating on somatic resemblance.

3. The selection pressure applied—the intensity and differential values.

4. The heritability of the characters in the foundation stock and in later generations. It would be preferable if measures of the relative genetic components—additive, dominance and epistatic deviations—were also available.

5. The amount of change in the environmental components of variation from time to time.

6. The averages and variations in each generation for the entire population, for the parents of the next generation and for their offspring.

7. The averages and variations in contemporaneous unselected controls derived from the same foundation stock as the selected groups and maintained in an environment similar to that of the selected groups throughout the experiment.

8. The effects of back selection when it is begun after different numbers of generations of selection in the high and the low lines.

9. The results of relaxation of selection after different numbers of generations of selection.

10. The extent of change in characters for which no conscious selection was practiced.

11. How much of the net selection practiced would have been expected to occur automatically, and how much was artificially imposed.

12. The results of crossing divergent lines with each other and with tester stock.

13. The effects of selection on the regression of offspring on parent.

Before the results of laboratory animal selection experiments can serve as useful guides in the solution of livestock selection problems it is obvious that the differences in mating habits, reproductive rates, amounts of deleterious inheritance etc., between the species must also be considered. Likewise, estimates of the genetic and non-genetic components of variation and of the phenotypic and genotypic correlations in the livestock characters must be known. Finally, the economic feasability of the procedures must be weighed.

What are the results of the laboratory animal selection experiments thus far reported? What contribution can they make to our understanding of the application of genetic theory to livestock selection problems?

The most extensive selection experiments in laboratory animals are those on bristle number in Drosophila [MacDowell (1915, 1917, 1920), Payne (1918, 1920), Sturtevant (1918), Mather (1941, 1942), Sismanidis (1942), Heuts (1948) and Rasmuson (1948)], those on length of wing and thorax in *Drosophila,* [Reeve and Robertson (1948)], hooding pattern in rats [Castle and Phillips (1914), Castle (1919)], sex ratio in rats [King (1918, 1919)], efficiency of food utilization in rats [Morris, Palmer, and Kennedy (1933)], white head spots in mice [Goodale (1937)], body weight in mice [Goodale (1938), MacArthur (1944, 1949)], ovarian response to a hormone in rats [Kyle and Chapman (1948)].

Collectively, these experiments (in many of which selection has been for both positive and negative deviates) have indicated that:

1. Selection with and without inbreeding has been effective in producing changes in the positive and negative direction over many generations. In some cases rather uniform increases in divergence between positive and negative groups have occurred for as many as 20 to 30 generations of selection. More commonly, however, the major changes have been found in the first generations of selection and in several experiments it would appear that there have been alternating periods of steady changes for several generations followed by generations of little or no change.

2. The ratios between the positive and negative line-changes from generation to generation (whether measured in absolute or relative terms) vary from experiment to experiment.

3. Back selection and relaxed selection in positive and negative lines have resulted in a rapid return to the original level in some cases but have had little or no effect in others. Effectiveness of back selection appears to be dependent on the number of generations of original selection preceding it in some cases but not in others.

4. Crosses between individuals from lines selected for positive deviations with those from lines selected for negative deviations have usually resulted in offspring intermediate between the parents.

5. Relative variation (coefficient of variation) within the selected lines has, as a rule, remained fairly constant throughout the generations of selection.

6. In several experiments changes correlated with those in the selected character have been noticed in characters for which no conscious selection was practiced.

7. In one experiment it was reported that starting to select after 5, 10, and 15, but not after 20, generations of brother and sister mating without conscious selection it was possible to establish high and low lines.

8. Specific chromosomes (autosomal and sex) have been identified as being the main source of the variation on which selection worked in several experiments.

9. In one experiment, reduction in heritability in later generations of selection appeared to occur.

10. A clearcut example of a generalized environmental influence limiting the effectiveness of selection in one direction and not the other is furnished by one experiment.

11. Several experiments illustrate the vast potential genetic variability which may be available for selection and effectively used by selection in a character relatively invariable in the foundation stock.

Most of the experiments from which these results were drawn lack much of the qualitative and quantitative information indicated as essential in the discussion of the features to be incorporated in the planning and analyses of laboratory animal selection experiments if their results are to have maximum transferability to other species. It is therefore extremely difficult to evaluate the results in terms which have specific applicability.

All that is clear, in general, from these experiments on selection in laboratory animals is that there are no obvious inconsistencies between

the results and genetic theory. The course of selection apparently takes many paths and no one pattern is sufficiently predominant to characterize all the results.

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For Economically Important Characters in Swine[1]

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A SYMPOSIUM is by definition the expression and discussion of ideas and opinions. It is very likely that these ideas and opinions will have been formulated under diverse conditions. Consequently, it seems only fitting and proper to outline briefly the particular conditions which have existed and which serve as a basis for the thoughts to be expressed.

The development of the lines of swine which have supplied the data from which I will make some rather broad, general statements as to the effectiveness of selection should be reviewed briefly. This information has appeared in publications but there is ample justification for reviewing it at this time, because the procedures which have been followed may be largely responsible for the results obtained.

There are at the present time, in the Minnesota project, five inbred lines of Poland China swine. Two of these lines have been maintained without the introduction of outside breeding, two of the lines have been developed by combining three inbred lines and the fifth line rests on a foundation of six inbred lines. During the course of the experiment, seven lines have been discarded because of poor performance. Three of the seven were culled before they were even inbred, one was culled when the inbreeding reached about twelve percent and three at about twenty percent. In other words, selection has not only been by individuals and litters but also by lines.

In addition to the five inbred lines of Poland China swine there are, in the Minnesota project, two inbred lines developed from crossbred foundations. They are: Minnesota No. 1, which rests on a cross of Danish Landrace and Tamworth, and Minnesota No. 2, developed from a cross of Canadian Yorkshire and inbred Poland Chinas from the Minnesota project.

This brief description of the inbred lines in the Minnesota project will serve as a background when the performance of lines is discussed later and gives a little idea as to the kind of genetic material with which we have been working. Two points should be emphasized in this

[1] Paper No. 2263, Scientific Journal Series of the Minnesota Agricultural Experiment Station in cooperation with the Regional Swine Breeding Laboratory, Ames, Iowa, Bureau of Animal Industry, U. S. Department of Agriculture.

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connection. First, the existing inbred lines of Poland Chinas are either selected lines—lines that have demonstrated that the stock from which they originated was superior stock—capable of performing quite satisfactorily when inbred—or are the result of combining three or more inbred lines, inbreeding again and selecting on a performance basis. This has made possible new gene combinations within the Poland China breed. Second, the two inbred lines developed from crossbred foundations again provided an opportunity for new gene combinations which, combined with performance testing, made possible the identification of superior segregates.

Selection of breeding stock has been strictly on a performance basis. Performance is measured by five factors. They are: (1) Fertility or number of live pigs farrowed, (2) survival or number of pigs weaned, (3) rate of gain from weaning to 154 days of age, (4) efficiency of feed utilization, and (5) score. This is true regarding individuals, litters and lines. Selection is a continuous process extending throughout the entire year.

Not all the litters farrowed are tested. An excess is farrowed to allow for selection or culling at weaning. The selection at weaning time is almost entirely on size of litter; but litters that are obviously lacking in thrift are culled. Gilts producing the best litters are kept over for one or two additional years and an attempt is made to repeat the original mating. In the seven inbred lines described, approximately 65% of the litters farrowed have been tested; the percent of pigs farrowed that were tested would be about eighty. This will vary from year to year and between lines. In the earlier years of the project there were about ten test litters per line. At the present time, there are approximately twenty test litters per line.

Litters selected for testing are placed in individual pasture lots ¼ acre in size, at time of weaning and the entire litter fed out to 154 days of age or a 98 day feed lot test. At this time each individual pig is scored for desirability of conformation. Boar pigs are castrated periodically throughout the feeding test resulting in a reduction in their numbers by about one-half by 154 days. The uncastrated boar pigs may be rather evenly distributed throughout the test litters or they may be found only in a few. Maximum selection for growth rate is possible only if a good number of boar pigs are left uncastrated until at least four months of age. Final selection of boars very often is not made until breeding time. Gilt selection at 154 days of age is primarily on rate of gain since fertility received special emphasis at weaning time. Score is also considered in gilt selection at this time as well as feed require-

TABLE 1

PERFORMANCE OF INBRED LINES OF SWINE AT THE MINNESOTA AGRICULTURAL EXPERIMENT STATION

		Fertility		*Survival*		*Weaning Wt., Lbs.*		*154 Day Wt., Lbs.*		*Rate of Gain, Lbs.*		*Feed Requirement* [1]	
Line	Fx, % 1948	3 yr. av. 1946–1948	1949	3 yr. av. 1946–1948	1949	3 yr. av. 1946–1948	1949	3 yr. av. 1946–1948	1949	3 yr.av. 1946–1948	1949	3 yr. av. 1946–1948	1949
M	78	7.0	6.3	5.4	5.3	32.0	31.0	140	130	1.09	1.0	329	410
V	42	7.6	8.1	5.2	6.6	32.2	31.7	173	185	1.44	1.55	318	309
A	37	7.6	7.4	6.0	6.9	34.0	33.2	187	194	1.56	1.65	318	305
B	31	7.3	7.5	4.1	4.3	27.4	28.5	172	166	1.45	1.39	301	305
C	23	6.2	7.3	5.2	6.1	28.9	29.0	176	170	1.48	1.44	327	358
#1	36	9.1	10.0	5.9	6.9	26.3	32.6	170	166	1.43	1.32	295	341
#2	28	7.7	8.3	5.3	6.4	30.4	30.6	162	168	1.33	1.39	318	300

[1] Pounds of feed required for 100 lbs. gain in the feed lot, a 98 day period.

ments. More females are saved for breeding than are wanted for farrowing. It has been found that 20 to 25 percent extra females is a sufficiently generous allowance for gilts which do not settle on first service, to be discarded, as well as the occasional gilt that does not remain thrifty or does not develop as well as expected.

From the outset the inbreeding program has been a flexible one; the inbreeding has not been advanced at any fixed rate or by any given procedure such as half-brother x half-sister matings. This procedure appeared to offer more room for maneuvering in selections and matings than a fixed program. The plan has been, however, to advance the inbreeding as rapidly as possible without sacrificing performance. To accomplish this, matings with a rather wide range in degree of closeness have been made, for the most part, within the different lines.

Perhaps the best measure of the effectiveness of selection is the performance of the lines at the present time. The performance of the 1949 spring farrowed crop is compared with the average of the three preceding years, 1946, 1947 and 1948 (table 1).

Just a word or two in conclusion. Environmental effects are, without question, the most difficult to control and appraise. While an attempt is made to maintain standard conditions, it is perfectly obvious that this is impossible. Year-to-year and station-to-station variations do occur and defy accurate appraisal.

Somewhat aside from the real purpose of the symposium and yet a matter of interest to all, is the development of lines. In order for selection to be effective there must be variation and to have lines that will stand a chance of making a contribution, there must be performance. In the final analysis the true worth or performance of a line will be measured or determined by its value in crossing. This has been a dominant philosophy in the Minnesota project.

For Economic Characters in Swine

G. E. DICKERSON

University of Missouri

THE expected rate of improvement from selection generally has been based on (1) the intensity of selection applied each generation, (2) the degree of heritability and (3) the interval of time between generations, considering each of the desired traits independently. As actually

applied in recent swine breeding experiments, selection has been based on some sort of a weighted average of the more important characters, and has been accompanied by mild inbreeding. The effectiveness of this selection can be evaluated from (1) the average time trend for each character within experimental strains, adjusted for the decline due to increased inbreeding, (2) comparison of strain crosses with representative purebreds and from (3) comparison of topcrosses with representative purebreds.

Time trends in performance are misleading as measures of genetic change to the extent that they are influenced by time trends in nutrition, disease, management, climate, or other factors. Nevertheless, they provide a measure of improvement achieved. A study[1] has been made of time trends in litter size and growth rate within strains in data from five projects of the Regional Swine Breeding Laboratory (49 strains, with an average of 9 seasons each). These traits were chosen because most of the deliberate selection had been based on them. On the average it was found that selection had produced no improvement in size of litters weaned or in weight per pig at five months of age. The unadjusted trends (linear regression on time) average —.21 per year in pigs weaned per litter, ranging from +.06 to —.43 pigs per year for the five projects; and —4.0 pounds per year in pig weight at five months of age, ranging from —1.5 to —8.2 pounds per year for the four projects from which unselected post-weaning growth data were available.

The average increase in percentage of inbreeding varied from 2.3 to 3.9 per year among the five projects. An earlier study of inbreeding effects alone, based on intra-season comparisons of inbreds and line crosses at four stations, indicated that for each increase of 10 percent inbreeding of dam *and* litter there was a highly significant decline of about .7 pigs per litter weaned and of 3.4 pounds in weight per pig at five months. These estimates for the influence of inbreeding alone were similar from the four stations, ranging from —.5 to —.9 for pigs weaned per litter and from —2.6 to —4.3 for pounds per pig at five months.

When the time trend in performance expected from the inbreeding-rise is subtracted from the actual intra-strain time trend, the adjusted time trend averaged 0.0 pigs per year for size of litters weaned, varying from —.16 to +.26 pigs per year between stations; and averaged —2.9 pounds per year for pig weight at five months, varying from —.6 to —7.0 per year between stations. Differences between stations may reflect differences in time trends for non-hereditary influences on per-

[1] To be published in more detail, separately.

formance. These time trends may be biased favorably because many of the strains that deteriorated most rapidly with inbreeding were discarded rather early and hence were given less weight than the strains retained in computing the average time trends. The average results indicate no general improvement in litter size and growth rate from selection within these closed strains unless nutrition, disease or management factors have been steadily deteriorating in these herds. Time trends in feed utilization and carcass composition were not analyzed. However, inbreeding has been shown to have little detrimental influence on either feed requirements or carcass composition. Selection for efficient feed utilization and for desirable carcasses has been largely indirect through selection for rate of gain and for conformation of live animals.

Too few carefully planned comparisons have been made of crosses among the inbred strains with representative purebreds, but the general level of linecross performance does not suggest that any major improvement has been achieved from selection during development of the lines. Rather extensive intra-herd comparisons of progeny from inbred boars of selected lines with those from representative purebred boars of the same breed have been made on farms in two projects. Here again, no noticeable superiority has been apparent in the genetic merit of the inbred strains for viability or rate of growth. Prolificacy of progeny has not been measured. Of course, some lines undoubtedly are superior to others, and more intense selection between lines based on cross performance during development of inbred strains presumably would improve performance levels of both linecrosses and topcrosses.

The apparently negligible improvement in litter size and growth rate was not due to failure to select for these characters. A cooperative study of the amount and kind of selection applied in developing these inbred strains is nearing completion. Data from four projects indicate that selection practised *each year* was equivalent to selecting breeding animals from litters that were above the average by .8 pigs farrowed and by 1.2 pigs weaned and to choosing individuals weighing about 4 pounds above average at weaning and 16 pounds above average at five months of age. However, the selection for litter size was little more than would have occurred automatically if the numbers of pigs selected from each litter had been proportional to the number of pigs available in that litter. It seems doubtful that purely automatic selection for larger litters would produce any genetic improvement in litter size. Of course, it is likely that much of the automatic selection for larger litters would have been offset by discrimination against pigs from the

larger litters because of their slower individual growth rates, unless there had been some deliberate selection for larger litters. Also, if there is any negative physiological relationship between rapid gaining and good suckling abilities, selection of the faster growing pigs within the larger litters as breeders would tend to cancel the effect of the selection for larger litters.

Neither can we ascribe the ineffectiveness of selection for litter size and growth to lack of heritable variation. Many studies have indicated that the average effects of genes (those linearly associated with genotype) account for 1/10 to 1/6 of the variation in litter size at weaning and for 1/5 to ¼ of the variation in weight of pigs at five months. In the controlled experiments with growth rate at Illinois and with feed utilization at Alabama, selection was definitely effective in separating the high and low lines. But the time trends in the growth rate experiment at least, suggest that most of the line difference was brought about by a decline in performance of the "low" line in response to selection in the reverse direction from that usually practised. Heritability is higher for body dimensions and for carcass composition and steady changes in these traits have been produced by selection. However, selection for such characters has been mild and has changed directions frequently, as contrasted with selection for viability and growth rate. Genetic-environmental interaction may be important as a cause of shifts in direction or intensity of selection for individual genes and consequent reduced effective heritability.

Some of the major facts pertaining to the effectiveness of selection for economic characters in swine may be summarized as follows:

(1) The portion of the observed variation which is linearly associated with genotype (*i.e.*, heritability) is considerable for most traits, but is lower for those characters for which selection has been appreciable and always in one direction (*e.g.* viability) than for characters for which selection has been mild or quixotic (*e.g.* conformation and composition of carcasses).

(2) The proportion of heterozygous loci or degree of inbreeding has its major influence on those highly important but lowly heritable characters for which selection has been consistently in one direction over long periods of time.

(3) Rather strong selection for larger litters and for faster growth within many mildly inbred strains has been only slightly effective, whereas selection has been effective in producing large changes in body dimensions and in carcass composition.

(4) Negative genetic-physiological associations between different desired characters have been indicated in several studies (*e.g.* between good suckling and efficient gaining abilities and between rapid growth and lean carcasses).

(5) Each of the existing breeds excels in only part of the desired characters, but crossbreds tend to equal or exceed the better parent breed in each respect and hence possess a clear advantage in total performance.

(6) The decline in performance with increased inbreeding has been essentially linear and quite similar for different strains and at different stations.

The facts cited suggest the following working hypothesis as a basis for further investigation of breeding methods for improving economic characters in swine: Most genes (or linked groups) affect many different vital processes and stages of development, some favorably, others unfavorably. Selection has stabilized at intermediate frequencies only those genes (or complexes) whose favorable effects are expressed and whose deficiencies or unfavorable effects are unexpressed in the heterozygous state. Genes at intermediate frequencies contribute more to genetic variability than genes approaching fixation or elimination, and hence account for a major portion of the observed hereditary variation in total performance. Homozygotes lack the favorable effects of the alternative allel(s) and selection favors heterozygotes over either of the alternative homozygotes.

In this sort of a genetic system, each character will be heritable to some degree, as measured by correlations between relatives, but positive genetic deviations for one character (or component of a character) will tend to be negative genetic deviations for other characters (or components). Selection for one character will tend to be detrimental to other characters, so that selection for total performance will be only slightly effective and hence relatively powerless to minimize the decline in performance from the loss of genes coincident with inbreeding.

Under heterozygote advantage, mean performance can never approach the potential maximum in any closed population or breed under selection, because more than one-half of the individuals are homozygous at any one locus and each individual is homozygous at more than half of the loci segregating in the breed as a whole. Maximum performance can be attained only in line crosses, by deliberately developing lines which are opposites in gene frequency at a large proportion of the loci exhibiting heterozygote advantage. Variability in proportion of heterozygous loci among crosses from any one generation will be small relative to the potential range because of the large number of loci involved. Hence, recurrent selection within each of the complimentary populations based on performance of strain-cross progeny will be necessary for sustained progress. Only limited progress would be expected from reliance on chance occurrence of favorable crosses among a single population of lines produced by prolonged inbreeding and intra-line selection. Of course, some selection based on intra-strain performance probably will be necessary to prevent fixation of genes of a semi-lethal character because of selection for the heterozygote in test-crosses.

Under the hypothesis stated, the performance of an inbred strain might be expected to improve under continued selection if the rate of further inbreeding were made negligible by expanding the population, using more sires and avoiding close matings. Selection favoring heterozygous genotypes would tend to pull back toward intermediate frequencies those genes which prior inbreeding had nearly fixed or eliminated, thus restoring part of the heterozygosity previously lost by inbreeding. This may account for apparently greater effectiveness of selection within strains after inbreeding is stabilized. The postulated heterozygote advantage would introduce a difference in the direction of change in gene frequency brought about by selection based on progeny test within strains as contrasted with selection based on family or especially individual performance (toward the more favorable homozygote and toward the heterozygote, respectively). However, the progeny test would be most useful as a means of selecting for opposite alleles in complementary strains based on strain-cross progeny performance.

It is emphasized that the proposed hypothesis applies primarily to characters such as viability, rate of growth, nursing or mothering ability, which have always possessed positive selective value, and to the effectiveness of selection for such traits during periods of time measured in decades rather than in centuries or evolutionary units.

For Economically Important Traits of Sheep[1]

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A STUDY was made of the amount of selection practiced and of the gains made from selection for certain traits of economic importance in a flock of range Rambouillet sheep to determine if this selection has been effective in producing genetic improvement in each of the various traits and in overall merit.

Fleece and body records were studied for 8,274 lambs, 1,215 yearling rams and 3,150 yearling ewes born from 1938 through 1948 in inbred Rambouillet lines. Selection differentials, or the difference between the average records of the parents and the average records of the entire population from which they were selected, were obtained for sires of

[1] Abstract of paper presented at the meetings of the American Society of Animal Production, Chicago, Ill., November 25, 1949.

lambs born from 1943 through 1948 and for dams of lambs born from 1946 through 1948.

Selection differentials were nearly all positive. Those for sires generally increased from 1943 to 1948 for staple length, type, face covering and weanling index. This appears to be due partly to increased accuracy of selection and partly to a decreasing generation length for sires. Expected genetic progress, obtained by multiplying selection differentials by heritabilities, has generally increased for the lamb crops from 1946 to 1948. The expected progress in weanling traits from selection of sires was far greater than from the selection of dams for staple length (40 to 70 times as great), face covering (8 to 12 times as great) and weight (4 to 11 times as great) and was generally 2 or 3 times as great for type and condition scores. From 81 to 89 percent of the expected genetic progress in the weanling index from 1946 to 1948 came from the selection of sires. The expected annual genetic progress in 1948 for weanling traits from selection of both sires and dams was 0.07 cm. in staple length, 0.84 pound in body weight, 0.018 score in type, 0.004 score in condition, 0.105 score in face covering and 0.047 score in neck folds.

Actual annual progress as shown by the regression coefficients of the traits on years from 1938 to 1948 was definitely positive for staple length, (0.07 cm. for weanling lambs) type score (0.062 score for weanling lambs) and neck folds (0.116 score for weanling lambs). Indications of improvement of condition and face covering were found. Body weight and fleece weight declined slightly in spite of positive selection differentials. Overall merit of weanling lambs increased from 1938 to 1948 as shown by a significant increase in the weanling index, although the rate of improvement was slightly less than that expected from the selection practiced.

Summary

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WE expect mass selection to cause the mean of the population to increase in each generation by an amount equal to the selection differential times the heritability. Some of us think we have seen signs that many populations do not actually change that rapidly. This seems to require an explanation.

First of all, it is possible that the dilemma may be only imaginary. Heritability may not be as high as we think. Selection may not have been as intense as we think. Perhaps the rate of progress actually is substantially as much as it should be. All three of these things need measuring more carefully.

The evidence on heritability seems generally fairly dependable and consistent from various experiments, if we take sampling errors into account and allow for the possibility of some environmental contribution still being in those estimates which are based on likeness between paternal half-sibs. The chief loop-hole seems to be that in these experiments we measure the heritability of a single characteristic. Two characteristics could both have moderately high heritability but be determined to a considerable extent by the same genes, each of which affects one of the characteristics favorably and the other unfavorably. Conceivably (although perhaps it is rarely so) this could happen to such an extent that, if we combined the two characteristics into a single measure of net merit, the heritability of that net merit would be almost zero. Apparently this amounts to the same situation as overdominance.

Selection may rarely be as intense as we think. Attempts to measure intensity of the selection actually practiced in whole herds or other populations have been few.

Possibly the mean of the whole herd or breed really is changing as rapidly as heritability and selection intensity make us think it should. Measurements of what actually is happening to the means of whole breeds, or of typical sections of whole breeds, have been few, sketchy, and uncertain of interpretation. Perhaps we ought to think of the old adage that when one is asked to explain how it is that witches can turn milk blue, the first thing is to find out whether witches really can turn milk blue!

Sometimes it is suggested that progress is slow or zero because mass selection was used, instead of progeny-testing or sib-testing. This explanation is inadequate. If mass selection will not work *at all,* neither will progeny-testing or sib-testing. Those do have an important place in efficient breeding plans and their proper use can speed up progress considerably under many conditions but, if the individual's own phenotype is no indication at all of its breeding value, then neither are the phenotypes of its offspring or of its sibs any indication of *their* breeding values, or of the breeding values of their parents or sibs. There are, however, several unexplored topics here concerning what may be expected if selection actually was on some combination

of individuality, ancestry, sibs, and progeny, but we compute the selection differentials and the expected results as if all the selection were mass selection. The answers to these questions seem more likely to make our dilemma worse than to explain it, but we can not be sure of that until they have been explored more thoroughly.

If the dilemma actually is a real one, what explanations are possible?

1. As more evidence accumulates from various species, overdominance seems more plausible as an important explanation. Yet, if that were the major explanation, it seems that the parent-offspring regressions would be very small, or even negative when the parents are the survivors of really intense selection. Likewise the resemblance between full sibs would be much higher than would be expected from the resemblance between parent and offspring or between half sibs. Something of the latter does occur often but it hardly seems enough to indicate that overdominance is the major factor. Where the full sibs usually occur in litters of contemporary individuals, as in swine, environment which full sibs have in common would be expected to make their resemblance noticeably high. Negative parent-offspring regressions, beyond what would be expected from sampling errors, are rarely if ever found.

2. Negative genetic correlations such as, for a hypothetical example, that the genes which make a sow a good milk producer might also make her slow in growth rate are a possibility. They seem to add up to the same thing as overdominance, except that we would not see the negative or zero parent-offspring correlations when we consider only one character at a time, although they would be found if we added all these characters together into a single measure of net merit and studied that. The matter of genetic correlations between two or more characters certainly does need more investigation. The present techniques for doing that require truly enormous numbers if the truth is to be bracketed within narrow confidence intervals. The use of identical twins is a possible exception to this, but the rarity of these seems to restrict their experimental use to cattle.

3. Possibly we select for a character at one stage of the life cycle and then undo the effects of this (unintentionally, of course) by selecting against it at another stage? As a possible example, rapid growth rate may make pigs look very good to us at ages of two to four months but make them overly coarse or fat or rangy at six months. Apparently this would cause the selection differentials to be nearly zero for each separate characteristic. In other respects it would come to nearly or quite the same thing as overdominance or negative genetic correlations. The topic needs clarifying.

4. Possibly we select for the characteristic in one herd or ecological niche and against it in another. This seems possible, and even likely in some characteristics, for a whole breed but it doesn't seem likely for data from a single experiment station. The latter kind of data furnish most of the usable evidence at present on the consequences of selection. Probably such local reversal of selection coefficients would make the net selection differential be zero in the whole population. The idea is one that is offered frequently to explain such problems as arise in the breeding of dual purpose cattle; namely, that a man at one time selects for beef qualities and against dairy qualities while the same man at another time, or another man at the same time, selects for milking qualities and against beef qualities.

5. Epistatic gene effects, such as occur when selecting for an intermediate as ideal, are a possibility. Apparently they would explain lack of continued progress in spite of actual positive correlations between parent and offspring or between other close relatives well enough, but it does not seem that they would explain the general prevalence of heterosis.

The primary question before us is whether the dilemma really exists. If it does not, most of these secondary problems or possibilities disappear. If the dilemma is real, does the trouble lie in our estimates of heritability? Or in measuring the selection actually practiced? Or are we overlooking something important about the real basis on which selections are made? Or are we mistaken in one or more ways about what the laws of inheritance would actually cause to happen under some of these situations?

COMPARATIVE NUTRITION OF FARM ANIMALS*

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THIS report is an outgrowth of the activities of the Committee on Animal Nutrition of the National Research Council. In 1942, the committee undertook the task of bringing up to date and continuously revising, as new information became available, the feeding standards for livestock. Subcommittees composed of specialists in each field were appointed, and a series of reports on Recommended Nutrient Allowances for Domestic Animals was issued by the National Research Council. After the reports for poultry, swine, dairy cattle, beef cattle, and sheep had been issued, Dr. L. A. Maynard of Cornell University critically reviewed them. He drew attention to certain discrepancies arising from differences in ideas of safety margins (which differentiate recommended allowances from minimum requirements) and to variations in nutritional philosophy and interpretation of data inevitable among several groups working independently.

The subcommittee chairmen were assigned the task of making a study of means of unifying and reconciling differences in the reports in process of revision. Previous feeding standards for the larger species had expressed allowances in terms of the amount of each nutrient per day for specified weights and production objectives. Poultry allowances, on the other hand, were for practical reasons always expressed as percentage of the nutrient in the ration or as amount per pound of feed. It was decided, therefore, to adopt uniform tables expressing the recommended allowances in both ways. When this was done, certain similarities and discrepancies in levels of intake and in trends with age became evident. To study the significance of these variations more critically, it was considered necessary to arrive at a more suitable basis for comparison. The authors were appointed to assume this task.

* A report of the Committee on Animal Nutrition, Agricultural Board, Division of Biology and Agriculture, National Research Council, Washington, D. C. A preliminary report of this study was published in the proceedings of the 5th International Zootechnic Congres, Paris, November, 1949.

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Review of Literature and Concepts Contributing to the Procedure Adopted

Sarrus and Rameaux (1839) proposed the theory that the rate of heat production of large and small animals is proportional to their respective body surfaces. This apparently was the first attempt to generalize quantitative nutritional knowledge. The history of the development of this concept, along with the controversies that still exist, was ably reviewed by Kleiber (1947). Since proportionality of basal heat production with weight$^{0.72}$ (Brody, 1932) and weight$^{3/4}$ (Kleiber, 1932) appears to fit the data better than expression of the surface law as weight,$^{2/3}$ weight $^{3/4}$ has been adopted by the Committee on Animal Nutrition as a suitable measure of metabolic body size.

Kleiber (1933) advanced the hypothesis that efficiency of feed utilization is independent of body size. This has been confirmed by Brody and Proctor (1935), Brody and Nesbit (1938), Kleiber and Mead (1941, 1945) and others. It is recognized that individuals of a species or even whole species may vary from the average in efficiency of feed utilization. Very broadly speaking, however, large and small species may have very similar efficiencies. This means that the total energy intake in relation to maintenance requirement or to basal heat production should be relatively constant. If this is true, then total feed capacity should be related to the same fractional power of body weight as basal metabolism.

Kleiber (1945) reviewed dietary deficiencies in relation to energy metabolism. Briefly, it may be stated that the amount of any dietary constituent taken by an animal is proportional to its concentration in the diet and the feed intake. The intake is determined mainly by energy requirement. If a given nutrient is needed in proportion to the animal's energy requirement, then its concentration in the diet determines the adequacy of the diet with regard to this nutrient. This appears to be true for most dietary essentials. Cowgill (1934) attempted to generalize requirements for vitamin B (thiamine) and arrived at a constant proportional to the 5/3 power of body weight. Many of the vitamin B complex entities had not been isolated at that time and his mathematical approach has been subject to criticism. Other workers (Williams, *et al.*, 1942; Melnick, 1942; Foltz *et al.*, 1944) express thiamine requirements directly in relation to calories in the feed. Similar data are available for riboflavin (Axelrod *et al.*, 1941; Williams *et al.*, 1943); for phosphorus (Theiler *et al.*, 1927); and for protein (Smuts, 1935).

Vitamin A is an exception to the general rule of proportionality

between requirement of an esential nutrient and energy requirement (Guilbert and associates, 1935, 1937, 1940; and Goss and Guilbert, 1939). These workers concluded that in mammals the minimum vitamin A requirement was directly proportional to the body weight.

Brody (1945), by suitably adjusting the time scale and expressing growth in percentage of mature weight, fitted the growth data of a wide

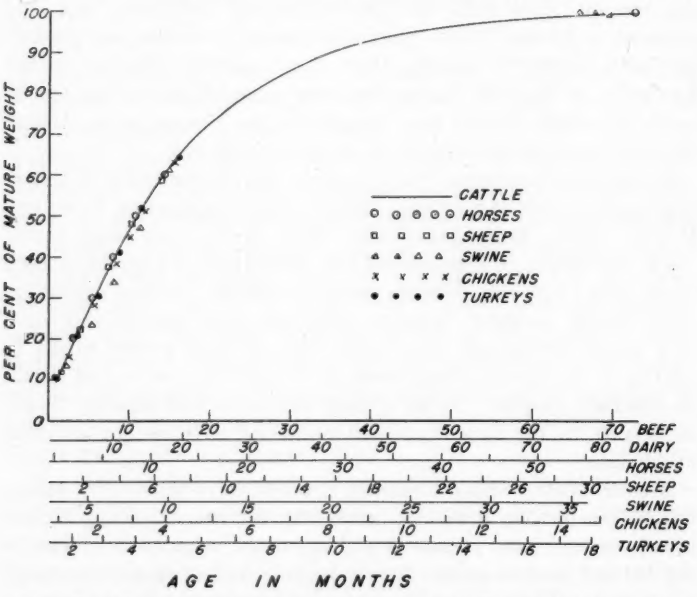

Figure 1. The weight-growth equivalence derived from the data on which the Recommended Allowances of Domestic Animals are based.

variety of species, including man after puberty, to a single curve. This concept of physiologically equivalent age has been used to compare the recommended allowances for the species covered by the National Research Council reports. These comparisons were made for each species at weights corresponding with 10, 20, 30, 40, 50, 60, and 100 percent of mature weight.

Presentation of Data

Growth and feed consumption data are based upon animals corresponding in size with the following breeds: Dairy cattle, Holsteins;

beef cattle, Herefords; sheep, medium down breeds or Rambouillet; swine, American meat types; turkeys, average bronze; chickens, White Leghorns.

In the recommended allowances for horses, data were given for mature weights to 1800 pounds. Values for animals having mature weights of 1000, 1400, and 1800 pounds were selected for this comparative study.

TABLE 1. PHYSIOLOGICALLY EQUIVALENT AGES AND BODY WEIGHTS

Species	Percent of Mature Weight						
	10	20	30	40	50	60	100
	Average Age in Months						
Horses, heavy weight	0.5	2.0	4.0	6.0	8.5	11.3	48
Horses, medium weight	0.5	2.5	4.3	6.7	9.5	13.0	45
Dairy cattle	1.8	4.8	7.1	10.0	13.4	17.4	84
Beef cattle	1.0	3.5	5.5	8.0	11.0	14.6	72
Horses, light weight	0.5	2.0	4.0	6.0	10.0	14.0	44
Swine	3.2	4.8	6.3	7.3	8.4	9.6	24
Sheep	0.5	1.2	2.5	3.5	4.5	6.5	24
Turkeys	1.4	2.1	2.8	3.4	4.0	5.0	11
Chickens	1.0	1.5	2.0	2.8	3.2	3.8	8
	Average Weight, Pounds						
Horses, heavy weight	180	360	540	720	900	1080	1800
Horses, medium weight	140	280	420	560	700	840	1400
Dairy cattle	140	280	420	560	700	840	1400
Beef cattle	130	260	390	520	650	780	1300
Horses, light weight	100	200	300	400	500	600	1000
Swine	50	100	150	200	250	300	500
Sheep	15	30	45	60	75	90	150
Turkeys	1.8	3.6	5.4	7.2	9.0	10.8	18.0
Chickens	0.4	0.8	1.2	1.6	2.0	2.4	4.0

Table 1 presents for the various species the physiologically equivalent ages and body weights as calculated from the committee reports. To test the validity of the physiological equivalence at the designated percentage of mature weight the curve shown in figure 1 was plotted. The beef and dairy cattle data fell on the same line. Because of the apparent adequacy of these growth data and the limited nature of the figures for other species, the cattle data were accepted as a tentative standard. Adjustment of the values for other species to the curve was accomplished by starting with 10 percent of mature weight to compensate for species variation in degree of maturity at birth, and by selecting an age of maturity which permitted a good fit with the tentative

TABLE 2. DAILY INTAKES OF TOTAL AIR DRIED FEED AND TOTAL DIGESTIBLE NUTRIENTS AT PHYSIOLOGICALLY EQUIVALENT BODY WEIGHTS

Species	Percent of Mature Weight						
	10	20	30	40	50	60	100
Total Daily Feed, Pounds							
Horses, heavy weight	—	13.0	16.0	18.6	20.7	22.3	19.5
Horses, medium weight	—	10.3	12.3	14.3	16.0	17.4	16.2
Dairy cattle	3.5	8.0	11.4	14.0	17.0	19.5	21.0
Beef cattle	—	—	11.7	14.5	16.7	18.7	19.5
Horses, light weight	—	8.3	9.7	11.0	12.3	13.5	12.6
Swine	3.0	5.3	6.8	7.5	8.3	8.8	—
Sheep	—	1.8	2.0	2.3	2.8	3.0	—
Turkeys	0.16	0.27	0.35	0.38	0.40	0.43	—
Chickens	0.066	0.091	0.111	0.129	0.140	0.151	0.158
Daily Feed Intake, Percent of Body Weight							
Horses, heavy weight	—	3.6	3.0	2.6	2.3	2.1	1.1
Horses, medium weight	—	3.7	2.9	2.6	2.3	2.1	1.2
Dairy cattle	2.5	2.8	2.7	2.5	2.4	2.3	1.5
Beef cattle	—	—	3.0	2.8	2.6	2.4	1.5
Horses, light weight	—	4.2	3.2	2.8	2.5	2.2	1.3
Swine	6.0	5.3	4.5	3.7	3.3	2.9	—
Sheep	—	—	4.5	3.7	3.7	3.3	—
Turkeys	8.9	7.5	6.5	5.3	4.4	4.0	—
Chickens	16.5	11.4	9.3	8.1	7.0	6.3	4.0
Daily Intake of Digestible Nutrients, Pounds							
Horses, heavy weight	—	8.1	10.0	11.6	12.9	13.9	12.2
Horses, medium weight	—	6.4	7.7	8.9	10.0	10.9	10.1
Dairy cattle	2.8	5.0	6.6	8.0	9.2	10.1	10.5
Beef cattle	—	—	6.6	7.7	8.8	9.3	9.5
Horses, light weight	—	5.2	6.1	6.9	7.7	8.4	7.9
Swine	2.3	4.0	5.1	5.6	6.2	6.6	—
Sheep	—	—	1.1	1.4	1.8	2.0	—
Turkeys	0.13	0.22	0.28	0.29	0.30	0.32	—
Chickens	0.053	0.073	0.089	0.097	0.105	0.113	0.120
Digestible Nutrients, Percent of Total Feed							
Horses, all weights	—	62.5	62.5	62.5	62.5	62.5	62.5
Dairy cattle	80	62	58	57	54	52	50
Beef cattle	—	—	58	55	53	51	50
Swine	75	75	75	75	75	75	—
Sheep	—	—	55	61	64	67	—
Turkeys	80	80	80	75	75	75	—
Chickens	80	80	80	75	75	75	76

standard. These mature ages were as follows: heavy horses, 60 months; swine, 36; sheep, 32; turkeys, 18; chickens, 15 months. The time of attaining mature weight is difficult to establish within narrow limits. The figures cited above appear to be more realistic than those submitted by the various committees. The scale adjustments, therefore, appear justified and the close fit of the data to the curve warrants the assumption of physiological equivalence at equal percentage of mature weight.

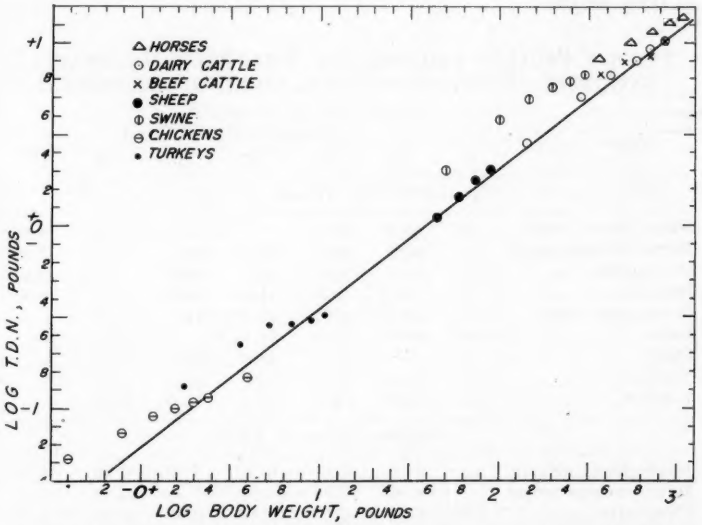

Figure 2. The logarithms of total digestible nutrient intakes are plotted against the logarithms of body weights showing that total feed capacity bears the same general relationship with weight ¾ as maintenance requirement and basal heat production.

The total daily feed consumption per animal and also the same data expressed in percentage of body weight are given in table 2. The percentages of digestible nutrients in the rations and the total daily intake of digestible nutrients per animal are also given in this table.

In figure 2, the logarithm of body weight for each species at various growth stages has been plotted against the logarithm of the corresponding total digestible nutrient intake, and these points are compared with the curve of proportionality with weight$^{3/4}$.

Table 3 shows the present recommended allowances for digestible protein, calcium, and phosphorus expressed in percentage of the total digestible nutrients (T. D. N.). This is done to allow comparisons on a basis more nearly equal than a percentage of total feed. Vitamin allowances expressed as amounts per pound of T. D. N. are given in table 4.

Because of the direct relation of vitamin A and carotene requirements with the body weight and the apparent uncertainty of the relationship of vitamin D, these data are given in table 5 as amounts per 100 pounds of body weight.

TABLE 3. PROTEIN, CALCIUM, AND PHOSPHORUS ALLOWANCES EXPRESSED AS PERCENT OF TOTAL DIGESTIBLE NUTRIENTS

Species	Percent of Mature Weight						
	10	20	30	40	50	60	100
	Digestible Protein, Percent of T.D.N.						
Horses, heavy weight	—	18.4	15.0	12.7	11.0	9.9	8.0
Horses, medium weight	—	18.8	14.9	12.7	10.7	9.2	7.9
Dairy cattle	20.7	14.8	12.3	10.6	9.6	9.0	7.6
Beef cattle	—	—	13.6	11.6	10.2	9.7	7.5
Horses, light weight	—	20.0	16.2	13.6	11.0	9.2	7.8
Swine	18.0	16.0	14.0	13.5	12.1	11.4	—
Sheep	—	—	15.5	12.9	10.5	10.0	—
Turkeys	24.0	24.0	20.0	20.0	20.0	16.0	—
Chickens	20.0	20.0	20.0	16.0	16.0	16.0	—
	Calcium, Percent of T.D.N.						
Horses, heavy weight	—	0.74	0.61	0.52	0.46	0.40	0.23
Horses, medium weight	—	0.66	0.59	0.52	0.46	0.41	0.23
Dairy cattle	0.99	0.84	0.65	0.49	0.41	0.35	0.30
Beef cattle	—	—	0.67	0.55	0.45	0.41	0.30
Horses, light weight	—	0.52	0.47	0.43	0.38	0.35	0.25
Swine	0.87	0.80	0.73	0.67	0.60	0.57	—
Sheep	—	—	0.56	0.49	0.42	0.41	—
Turkeys	2.5	2.5	2.5	2.67	2.67	2.67	—
Chickens	1.25	1.25	1.25	1.33	1.33	1.33	1.07
	Phosphorus, Percent of T.D.N.						
Horses, heavy weight	—	0.60	0.50	0.45	0.41	0.37	0.26
Horses, medium weight	—	0.55	0.51	0.46	0.41	0.37	0.26
Dairy cattle	0.59	0.55	0.50	0.42	0.37	0.33	0.30
Beef cattle	—	—	0.50	0.44	0.38	0.35	0.30
Horses, light weight	—	0.45	0.42	0.40	0.37	0.35	0.27
Swine	0.56	0.53	0.49	0.44	0.40	0.38	—
Sheep	—	—	0.38	0.33	0.27	0.26	—
Turkeys	1.25	1.25	1.25	1.33	1.33	1.33	—
Chickens	0.75	0.75	0.75	0.80	0.80	0.80	0.67

TABLE 4. VITAMIN ALLOWANCES EXPRESSED IN AMOUNTS PER POUND OF T.D.N.

Species	Percent of Mature Weight						
	10	20	30	40	50	60	100
Carotene Mg. Per Pound of T.D.N.							
Horses	—	2.2	2.7	3.1	3.5	3.9	7.4
Dairy cattle	3.0	3.4	3.8	4.2	4.6	5.0	7.9
Beef cattle	—	—	3.5	3.9	4.3	4.8	8.2
Swine	0.87	1.0	1.2	1.4	1.6	1.8	—
Sheep	—	—	2.5	2.6	2.5	2.7	—
Turkeys	3.0	3.0	3.0	3.2	3.2	3.2	—
Chickens	1.5	1.5	1.5	1.5	1.5	1.5	1.6
Vitamin A, I.U. Per Pound of T.D.N.							
Horses	—	1330	1620	1860	2090	2330	4400
Dairy cattle	1500	1700	1900	1800	2300	2500	4000
Beef cattle	—	—	1700	2000	2100	2400	4100
Swine	640	690	790	920	1040	1190	—
Sheep	—	—	1230	1285	1250	1350	—
Turkeys	5000	5000	5000	5000	5000	5000	—
Chickens	2500	2500	2500	2500	2500	2500	2680
Vitamin D, I.U. Per Pound of T.D.N.							
Dairy cattle	150	170	190	—	—	—	—
Swine	65	65	65	—	—	—	—
Sheep	—	—	123	129	125	135	—
Turkeys	1000	1000	1000	1065	1065	1065	—
Chickens	225	225	225	240	240	240	—
Thiamine, Mg. Per Pound of T.D.N.							
Swine	0.67	0.67	0.67	0.67	0.67	0.67	—
Chickens	1.12	1.12	1.12	1.20	1.20	1.20	—
Riboflavin, Mg. Per Pound of T.D.N.							
Swine	1.1	1.1	1.1	1.1	1.1	1.1	—
Turkeys	2.5	2.5	—	—	—	—	—
Chickens	2.0	2.0	2.0	1.2	1.2	1.2	—
Niacin, Mg. Per Pound of T.D.N.							
Swine	6.7	6.7	6.7	6.7	6.7	6.7	—
Chickens	10.0	10.0	10.0	10.0	10.0	10.0	—
Pantothenic Acid, Mg. Per Pound of T.D.N.							
Swine	6.3	6.3	6.3	6.3	6.3	6.3	—
Chickens	6.2	6.2	6.2	6.7	6.7	6.7	—

TABLE 5. ALLOWANCES OF CAROTENE, VITAMIN A, AND VITAMIN D PER 100 POUNDS OF BODY WEIGHT

Species	Percent of Mature Weight						
	10	20	30	40	50	60	100
	Carotene, Mg.						
Horses	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dairy cattle	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Beef cattle	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Swine	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Sheep	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Turkeys	21.7	18.3	15.5	12.1	10.0	8.9	—
Chickens	11.3	7.8	6.2	9.1	7.9	7.1	4.8
	Vitamin A, I.U.						
Horses	3000	3000	3000	3000	3000	3000	3000
Dairy cattle	3000	3000	3000	3000	3000	3000	3000
Beef cattle	3000	3000	3000	3000	3000	3000	3000
Swine	2600	2600	2600	2600	2600	2600	2600
Sheep	3000	3000	3000	3000	3000	3000	3000
Turkeys	36000	30600	26000	20000	16700	14800	—
Chickens	19800	13700	11100	14500	12600	11300	8000
	Vitamin D, I.U.						
Dairy cattle	400	400	400	—	—	—	—
Swine	270	250	220	190	170	150	—
Sheep	300	300	300	300	300	300	—
Chickens	2980	2050	1670	1450	1260	1130	—

Discussion

Total Feed Capacity. Figure 2 shows that, broadly considered, all of the species have approximately equal relative feed capacities, because the total nutrient intake is shown to be roughly proportional to the same fractional power of body weight as the basal metabolism and the maintenance requirement. If all species conformed exactly to the curve representing weight$^{3/4}$, then one would expect all to be equally efficient in converting feed nutrients into body substance.

The recommended allowances for chickens, turkeys, and swine represent maximum feed consumption. This apparently is also the case for horses. The allowances for dairy and beef cattle are for normal growth with only limited fattening, while the level for sheep is intermediate. The beef cattle, dairy cattle, and sheep data fall on or very close to the curve and offer further confirmation of equal efficiency of these species in feed use for growth. Beef calves that are full fed early in life fall

somewhat above the curve, but not so high as poultry and swine. The high relative feed capacity shown for swine is in line with their greater efficiency of feed utilization for growth. In all cases the data approach the curve of weight$^{3/4}$ with advancing maturity.

Digestible Protein. The data in table 3 for horses, dairy cattle, beef cattle, sheep, and swine are sufficiently similar to suggest that they may well be identical. The hypothesis is advanced, therefore, that the

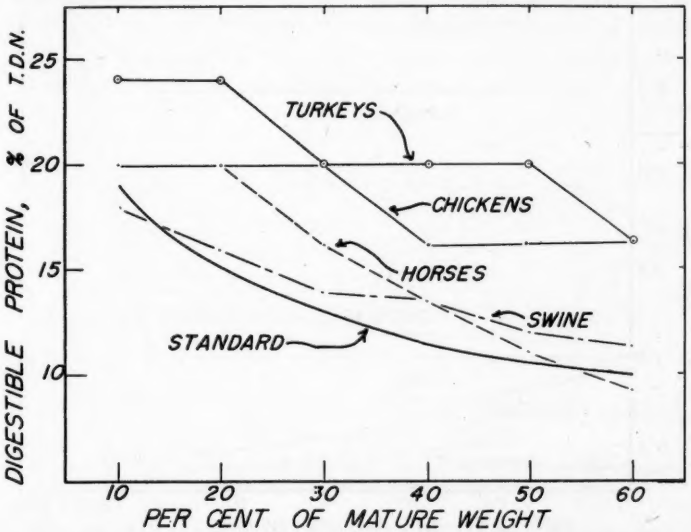

Figure 3. The "generalized standard" curve for digestible protein levels in comparison with the recommended allowances for poultry, horses, and swine.

ratio of required protein to available energy intake is the same for the various species at physiologically equivalent growth stages. The ratio changes, however, with alerations in the composition of growth increments with advancing age.

Figure 3 is presented to illustrate more clearly the similarities and differences in the recommended allowances. On the basis of the data under consideration, the authors have proposed and shown a "generalized standard" curve, hereinafter referred to as the "G. S." curve. This fits closely with the average ruminant data. The swine data of Woodman and Evans (1948) fall below the "G.S." curve at 20 percent of maturity and on the curve at 30 percent. The British standard for swine cited

by Watson (1949) varies from 10 to 13 percent below the "G. S." curve, while the Swedish data (Jespersen, 1949) are 10 percent below at 10 percent maturity and somewhat above at 40 percent maturity. Expressed on the same basis, Morrison's minimum protein and T. D. N. figures give values 115 to 130 percent of the "G. S." curve. On the other hand, if his minimum protein figures are expressed as percentage of maximum T. D. N., his values agree closely with the "G. S." curve up

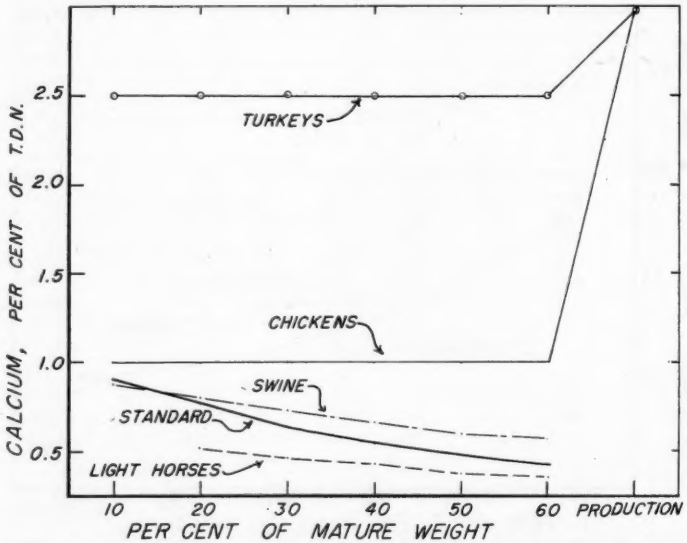

Figure 4. The "generalized standard" curve for calcium compared with recommended allowances for light horses, swine, and poultry.

to 40 percent maturity. Thereafter, they are somewhat higher. The assumption that the "G. S." curve would suffice for swine, therefore, appears reasonable. At the 10 percent stage, the allowances for chickens, swine, and dairy cattle vary from 18 to 20 percent of the T. D. N. (table 3). The recommended allowances for turkeys are higher. Because of practical considerations involved with avoiding several changes in composition of rations for growth stages that are passed within comparatively short periods of time, protein allowances for poultry do not decline with advancing age in the same manner as those for other species. Generally, poultry rations fall into three categories—starting feeds, growing feeds, and laying rations.

The high protein level for turkeys and for chickens at later growth

stages warrants further investigation and explanation. In the United States, poultry consume about 30 percent of the total protein concentrates and more than any one of the other species. If poultry are found to conform more closely in actual requirement to the curves of the other species, the saving of protein feeds could be very great.

Data for horses of 1000 pounds mature weight are plotted in figure 3. Table 3 shows lower values than these for heavy horses and intermediate figures for the medium size. Since the percentage for protein in the T. D. N. for all different sized horses is significantly higher, at least up

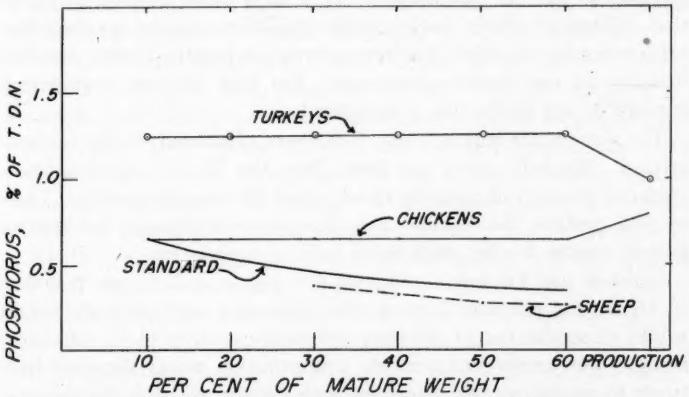

Figure 5. The "generalized standard" curve for phosphorus compared with recommended allowances for sheep and poultry.

to 50 percent of maturity, than for other farm animals, a re-examination of these recommended allowances is suggested. Further research doubtless is required to settle the question.

Calcium and Phosphorus. Considering the nature of the data on which they are based, the calcium and phosphorus allowances for heavy horses, dairy cattle, beef cattle, sheep, and swine are so similar that identical requirements are again indicated (table 3).

"G. S." curves were therefore established on the basis of the averages for the species named. In figure 4, the variations from the "G. S." curve of the calcium allowances for swine, light horses, chickens, and turkeys are graphically presented. The calcium allowances for heavy horses fit closely with the "G. S." curve; those for medium horses are intermediate. The reasons for these differences within a species are not apparent.

In figure 5, the recommended allowances for sheep, turkeys, and chickens are compared with the "G. S." curve for phosphorus. Horses

(table 3) again show systematic differences which are more conspicuous at early growth stages than later, the allowances being highest for heavy and lowest for light horses.

The recommended allowances for both calcium and phosphorus for turkeys and chickens remain constant with advancing age, while they decline with other species. The calcium levels represent the maintenance of a uniform, arbitrary calcium-phosphorus ratio which may be justified on practical grounds but is not consistent with known changes in actual demands for these elements as the body grows, develops, and alters in composition. The calcium level for turkeys is so conspicuously high that deleterious effects under certain conditions appear possible. One explanation for the high phosphorus levels for poultry is their reported inability to use phytin phosphorus. The high calcium requirement imposed by egg production is recognized.

The phosphorus requirements of chickens calculated by the factorial method (Mitchell, 1947) are lower than the "G. S." curve at early stages of growth but coincide closely after 30 percent maturity. Thus, as with protein, the calcium and phosphorus allowances for poultry growth require further verification and/or explanation.

Carotene and Vitamin A. Expressed in mg. or in I. U. per pound of T. D. N., the amounts increase with advancing age and body weight within all species except chickens and turkeys (table 4). If the safety margins were made uniform, the concentration would decrease from larger to smaller species, consistent with the concept that requirements are directly proportioned to body weight rather than to energy metabolism. Table 5 shows the same data expressed per unit of body weight. The recommended level for ruminants is 6.0 mg. per 100 pounds of body weight or 132 micrograms per Kg. This is five times the accepted minimum level (1.5 mg. per 100 pounds body weight) for optimum growth and for freedom from clinical symptoms but permits only a slight margin of safety for reproduction. The swine and horse committees considered 4.0 and 5.0 mg. per 100 pounds live weight, respectively, to have sufficient margin of safety. From a practical viewpoint these differences are somewhat academic, but the basic data do not appear to warrant this lack of uniformity.

Except for the unpublished data of Kratzer and Guilbert, carotene and vitamin A requirements of poultry have been studied only in terms of concentration in the diet. In poultry, as in mammals, the requirement is directly proportional with body weight. The feed intake in relation to body weight changes rapidly in these fast-growing species; therefore, it is difficult to determine exactly the requirements from this type of

experiment. Expressed as concentration in the feed, the allowance for turkeys is double that of chickens. This is justified because feed consumption per unit of body weight of turkeys at the earlier stages of growth is about one half that of chickens of comparable age.

The recommended carotene level for chickens at 60 percent of mature weight approaches that for mammals. The mammalian level is about minimum for slight liver storage, optimum dark adaptation (man), and reproduction. This same level for chickens may not have so much margin of safety because the present evidence is that the requirement is somewhat higher for birds than for mammals. If this is true, the physiological explanation should be sought in further research.

The corresponding vitamin A levels (table 5) for poultry are conspicuously divergent from those for animals. This derives from the assumed equal value (expressed in International Units) of carotene and vitamin A for these species. Kratzer and Guilbert (unpublished data, California Agricultural Experiment Station) conducted experiments on minimum requirements of turkeys by individual dosing with levels maintained constant with body weight. According to their preliminary and tentative results, the minimum level for turkeys is in the order of 100 I. U. of performed vitamin A per Kg. or 4500 I. U. per 100 pounds of body weight. The corresponding level for carotene expressed in I. U. (0.6 microgram beta carotene=1 I. U.) appears to be about five times that for performed vitamin A, a level which corresponds with the relative values for mammals (Guilbert, Howell, and Hart, 1940).

The requirements for poultry need further study upon the basis of the fundamental relation with body weight. The recommended concentrations in the ration may then be calculated to maintain adequate margins of safety at all stages of development.

Vitamin D. The values in table 4 show no systematic trends on the basis of concentration in the ration. Reference to the same data in table 5 shows that for cattle and sheep the requirement was regarded as constant in relation to body weight; for swine and poultry the allowances are a constant proportion of the ration. Difference in quantitative requirement of species is recognized, as well as species variation in the efficiency with which various forms of vitamin D are utilized. The lack of agreement suggests, however, that the quantitative data are not entirely satisfactory and that a suitable basis for generalization requires further research.

Thiamine. The data (table 4) for swine and poultry are constant in concentration in the ration. The fundamental relationship to carbohydrate metabolism has been recognized and this is necessarily related

to total feed or energy intake. In addition to the data from the N. R. C. recommended allowances for man and domestic animals, table 6 includes figures for the dog and rat.

Considering variations in margins of safety, the thiamine figures indicate identical requirements in proportion to caloric intake, so long as the proportion of carbohydrate and fat remain relatively constant. Extending the data within species to growth stages not covered by experiment, or even predicting the requirements of other species as well as a uniform recommended allowance, appears justifiable.

Riboflavin. For chicks to 30 percent maturity, the recommended allowances have a larger safety factor but otherwise are constant in concentration in the diet and at later stages are similar to those for

TABLE 6. THE THIAMINE AND RIBOFLAVIN ALLOWANCES OF VARIOUS SPECIES

	Thiamine	Riboflavin	
	Mg/1000 Calories	Mg/1000 Calories	ug./Kg. of Body Weight
Adult man	0.4–0.5	0.4–0.75	26
Children	0.5	0.72–0.75	33–41
Swine	0.37	0.60	53–113
Turkeys	—	1.38	—
Chickens	0.61–0.66	0.66–1.10	124–586
Dog	0.4[1]	0.50–1.00	—
Rat	0.4[1]	0.5–0.8[2]	205–330[2]
Fox	0.4[1]	—	—

[1] This value is double the minimum requirement found by Arnold and Elvehjem (1938, 1939) and Harris and Loosli (1949) and corresponds with the human allowance which also provides a 100 percent margin of safety.
[2] Estimated from data of Edgar *et al.* Biochem. Jour. 31; 879.

swine (table 4). The turkey values are fragmentary and apparently assumed to be 25 percent greater than those for chickens.

Because of the statement in the recommended dietary allowances for humans, that riboflavin allowance should be based, not on calorie consumption but rather on body weight or possibly on some function thereof, the data for various species are presented in table 6 in amounts per 1000 Calories and also per Kg. body weight. Expressed in relation to caloric intake, the agreement is comparatively close between those species which vary widely in body size and hence in food intake per unit of weight. On the other hand, the allowances expressed in amount per unit of body weight are widely divergent. Moreover, the data show that for humans 20 ug. per Kg. or less is adequate while for swine 22 ug. per Kg. is definitely deficient.

For adult man the recommended riboflavin allowance is not increased as caloric intake is increased from 2400 to 4500 Calories per day. The allowances for children remain constant at 0.72–0.75 mg. per 1000 Calories. When expressed in relation to body weight these same allowances work out at 25 to 85 ug. per Kg.

The data presented offer strong evidence that riboflavin requirements may also be generalized in relation to energy intake or to body weight[3/4].

Niacin. The allowances for swine and chickens, similarly to those of the other vitamin-B complex entities cited above, are constant with feed intake. The allowances for man, swine, and chickens expressed as mg. per 1000 Calories, are 5.5 to 7.7 mg., 3.7 mg., and 5.5 mg., respectively.

Pantothenic Acid. Data were available for swine and chickens only. The differences shown in table 3 appear insignificant. The allowances are consistent with feed intake.

Summary and Conclusions

The data presented appear to warrant the following generalizations: In a broad sense, the total feed capacities of the various species are proportional to the same fractional power of body weight as maintenance requirement and basal heat production. There is no fundamental difference, therefore, between large and small animals in efficiency of feed utilization for growth that depends upon body size as such. Species or individuals that have higher than average relative feed capacity (feed intake in relation to basal heat production) during early growth stages tend to be higher in efficiency of feed utilization.

At physiologically equivalent ages the requirements, as reflected by recommended allowances, for protein, calcium, and phosphorus appear to be similar for the various species. The ratio of these nutrients to energy intake, however, changes with alterations in the composition of growth increments with advancing age.

Thiamine, riboflavin, niacin, and pantothenic acid allowances remain constant with energy intake irrespective of peaks of bone, muscle, and fat growth at different stages of development. This is consistent with their functions in enzyme systems associated directly or indirectly with metabolic rate. This generalization renders justifiable the interpolation and extrapolation of data for vitamin B complex entities within and between species. In this connection, it is interesting to note that the choline requirement of the German cockroach (Neland and Baumann, 1949) is 0.2 to 0.4 percent of the diet as compared with about 0.2 percent for turkeys and 0.1 percent for chickens!

Vitamin A and carotene requirements for mammals are equal and directly proportional to body weight. The requirement for poultry is also directly related to body weight, but apparently the requirement is somewhat higher than that for mammals. The physiological explanation of this requires further research. Vitamin A and carotene expressed in International Units on the conventional basis do not have equal biological value for either poultry or mammals. They are equivalent in the rat only at the level of dosage at which the *Unit* was originally defined. The higher the level of intake, the greater the divergence in efficiency

TABLE 7. GENERALIZED RECOMMENDED ALLOWANCES

Nutrient	Percent of Mature Weight					
	10	20	30	40	50	60
Digestible protein, % T.D.N.	19.0	15.0	13.0	11.5	10.5	10
Calcium, % T.D.N.	0.90	0.76	0.65	0.55	0.46	0.43
Phosphorus, % T.D.N.	0.65	0.56	0.47	0.42	0.37	0.34
Vitamin A, I.U./100 lbs. body weight[1]	3000	3000	3000	3000	3000	3000
Carotene, Mg/100 lbs. body weight[1]	5.0	5.0	5.0	5.0	5.0	5.0
Thiamine, Mg/lb. T.D.N.	0.9	0.9	0.9	0.9	0.9	0.9
Riboflavin, Mg/lb. T.D.N.	1.4	1.4	1.4	1.4	1.4	1.4

[1] Mammalian species.

of utilization of carotene compared with performed vitamin A. Much of the confusion arising from this situation would be obviated if evaluation in units was discarded and requirements were expressed in weights of the pure substances.

No definite relation of vitamin D requirement of allowances with either body weight or feed consumption is evident from the studies thus far available.

To give concrete form to these generalizations, and more specifically to guide the direction of future research to test their validity, generalized recommended allowances are brought together in table 7. These basic figures for growth, or such modifications as future research may reveal, can be translated into feeding standards suitable for practical use and constitute a consistent body of quantitative nutritional data. The need for further research evidenced by conspicuous diversion of allowances from the general trend has been cited and discussed in the text. For example, experiments on protein requirements could be set up to test not only the trend of the curve, but also its quantitative validity.

Refinements in the bases for comparison and extending the study to other species are in order. Determination of the relationships of minimum requirements and translating these into recommended allowances upon the basis of uniform and adequate safety margins should further reduce variability. The feasibility of expressing the relationship of the various nutrients with metabolizable energy rather than with total digestible nutrients should be explored. Although introducing complications, this should bring the data for ruminants and non-ruminants in closer accord. After accounting for both urinary and methane losses in the ruminant, however, differences involving specific dynamic effect still remain a complicating factor.

It can be argued that nutrient requirements during growth are related not only to age but also to percentage rate of growth and, therefore, the concentration of nutrients in relation to energy should be different for fast and slow growing species. This argument breaks down, however, if physiologically equivalent age means that the composition of growth increments are closely similar for the several species. The data show essentially equal ratios of total energy intake to basal heat production or maintenance requirements between small fast growing and large slower growing species. An animal which has a high percentage rate of growth at any specified physiological age has a high energy intake and a correspondingly high daily intake of the associated nutrients. Thus, at 10 percent of maturity protein allowance for both chickens and dairy calves is 20 percent of the T. D. N. (table 3). The daily feed intake (table 2) of the slower growing calves is 2.5 percent of body weight, for the fast growing chicks, 16.5 percent! In this connection Mitchell (1945, 1950), in his reviews of comparative nutrition, cites the dominance of the growth requirements of a rat having a percentage growth rate of 3.0 with the subordinance of growth to maintenance in a juvenile child having a percentage growth rate of 0.03. Previously it has been indicated that the growth of man does not fit the curve of physiologically equivalent age until after puberty. The foregoing discussion applies to the growth of species which fit with the physiologically equivalent age curve.

The authors have been impressed by the paucity of usable quantitative data which can be gleaned from the (wealth of) literature in the field of nutrition. All too frequently data are given for laboratory animals in daily doses for an animal having unspecified feed consumption and/or body weight. In the preoccupation of seeking new nutritional factors, it would appear desirable to pause when they are found to determine their fundamental relations to other nutrients, to body

weight, or to caloric intake, and the quantitative requirements in a systematic procedure.

Progress in all science has consistently demonstrated that we are living in an orderly universe and that the basic laws apply generally if one can but find the means for doing research and expressing results on comparable bases. Progress in comparative nutrition offers ample evidence that this field is no exception, that each species is not a special creation functioning in its own peculiar manner, but rather that the similarities are far more striking than the differences.

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VITAMIN B_{12}, APF CONCENTRATE, AND DRIED WHEY FOR GROWING-FATTENING PIGS[1, 2]

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THE essentiality of vitamin B_{12} in swine rations has been demonstrated by many investigators. Using "synthetic" milk rations, Anderson and Hogan (1950), Nesheim (1950) and Neumann *et al.* (1949) showed the need of vitamin B_{12} and reported on the quantitative requirements of baby pigs. Published experimental work on the vitamin B_{12} requirement of growing and fattening pigs has been limited. Using a crude vitamin B_{12} concentrate, Lepley (1950) of the Iowa Station reported that the tentative vitamin B_{12} requirement for growing and fattening pigs fed a natural corn-soybean oil meal ration was approximately 10 micrograms per pound of mixed ration.

Work conducted by Krider *et al.* (1949) and Dyer *et al.* (1949) indicated a growth factor or factors in dried whey for weanling pigs. They found that the addition of 2 percent dried whey to rations containing meat and bone scraps increased the growth rate of pigs significantly. Results of these trials as well as chick trials reported by Hill and Branion (1950) and Menge (1950) may have contributed to some research workers' belief that the so-called "whey factor" might be a portion of the APF complex.

According to Catron (1950) certain APF concentrates are now known to contain variable amounts of vitamin B_{12}, primary antibiotic residues and other fermentation residues.

The objectives of this study with weanling pigs fed in concrete drylot were: (1) to attempt to establish further the practical vitamin B_{12} requirement of growing-fattening pigs fed corn-soybean oil meal rations using a chick-assayed vitamin B_{12} concentrate and crystalline vitamin

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B_{12} as a positive control; (2) to determine whether the "whey factor" is a portion of the APF complex; and (3) to determine if a certain APF supplement supplied growth factor(s) in addition to vitamin B_{12} and the so-called "whey factor."

Method of Procedure

Ten pigs were allotted into each of nine lots on the basis of previous treatment, weight, age, condition, litter and sex. The pigs used in this study were not depleted of vitamin B_{12}. They were housed in pens of equal size with access to outdoor pens when weather permitted. The pens were cleaned daily to minimize coprophagy and reduce bacterial synthesis. Feed and water were offered *ad libitum*. All pigs were wormed with the basal ration containing one percent sodium fluoride for the first twenty-four hours of the experiment. The pigs were then started on the various ration treatments.

The basal ration was composed of ground yellow corn, blended expeller soybean oil meal obtained from three sources, minerals including the following trace elements: Mn, Fe, Cu, Co, Zn and K, and a vitamin premix that supplied 500 mg. choline, 15 mg. niacin, 10 mg. pantothenic acid, 2.5 mg. riboflavin, 1.5 mg. alpha tocopherol acetate, 200 units of vitamin A, and 400 units of vitamin D_2 per pound of total ration. Pigs in lots 2, 3, and 4 received the basal ration including a vitamin B_{12} concentrate fed at levels to provide 5, 10 and 15 micrograms of vitamin B_{12} per pound of ration respectively. Based on chick assay this concentrate contained 12 mg. of vitamin B_{12} per pound. Lot 5, which was used as a standard reference, received 15 micrograms of crystalline vitamin B_{12} per pound of ration. The addition of 2 percent dried whey was made to the basal ration for the pigs in lot 6. This same ration including the vitamin B_{12} concentrate added at a level to supply 15 micrograms of vitamin B_{12} per pound of ration was fed to the pigs in lot 7. The pigs in lots 8 and 9 received the basal ration including 0.44 and 0.66 percent APF supplement (Lot 26) respectively. Based on chick assay these two levels of APF supplement supplied 10 and 15 micrograms of vitamin B_{12} per pound of ration, or the same level of vitamin B_{12} as was fed to pigs in lots 3 and 4. The average aureomycin content of APF supplement (Lot 26) was 2386 mg. per pound. This provided 10.5 mg. of aureomycin per pound of mixed ration for pigs in lot 8, and nearly 16 mg. per pound of ration for the pigs in lot 9.

The beginning rations were calculated to contain 18 percent protein.

At average lot weights of 75 and 150 pounds, the protein levels were lowered to 15 and 12 percent respectively by increasing the amount of corn and decreasing the soybean oil meal. Quantities of the other ingredients remained the same.

The pigs were weighed at two-week intervals during the course of the experiment. They were removed from the experiment individually when they reached the final weight of at least 200 pounds. Feed records were kept by lot treatment.

Back fat measurements were taken on four barrows from each of the three lots; I, V, and IX to study the effects of vitamin B_{12} and APF supplements on back fat deposition. Depth of back fat was measured over the back of the shoulder, over the middle of the back and over the middle of the loin. A one-half inch incision was made through the skin about one inch off the midline of the body and a narrow metal ruler was pressed through the fat down to the *longissimus dorsi*. These measurements were taken on the live barrows by a method proposed by Hazel (1950) who reported a correlation of 0.83 between the average of the three live-hog measurements and the three measurements taken on the carcasses.

Statistical analysis of average daily gains and of average back fat depths were made according to procedures outlined by Snedicor (1946).

Results and Discussion

Average daily gain, average daily feed consumed, feed required per 100 pounds gain and number of days required to reach final weight are summarized in table 1. Average daily gains were improved by the addition of different levels of the vitamin B_{12} concentrate or 15 micrograms of crystalline vitamin B_{12}. The differences in gains approached significance at $P=.05$. This is in close agreement with work reported by Beeson and Bloss (1949) and Luecke *et al.* (1950).

The analysis of variance showed that the average daily gain was increased 0.16 pounds which was significant at ($P=.05$) by the combined addition of 2 percent dried whey and the vitamin B_{12} concentrate to the basal ration (lot 7). Highly significant increases of 0.27 and 0.37 pound in daily gains ($P\leqq.01$) were obtained by the addition of either 0.44 percent (lot 8) or 0.66 percent (lot 9) APF supplement. There was no significant difference in average daily gain between the two groups fed the different levels of APF supplement. The daily gains of pigs fed 0.66 percent APF supplement (lot 9) was significantly greater ($P=.05$) than pigs fed 2 percent dried whey and the vitamin B_{12} concentrate (lot 7).

The following lot treatments comprised a factorial design experiment and were analyzed accordingly: lot 1, the basal ration; lot 4, basal including 15 micrograms of vitamin B_{12}; lot 6, the basal including 2 percent dried whey; and lot 7, basal including 2 percent dried whey and 15 micrograms of vitamin B_{12}. It was found that the effect of the addition of dried whey to the basal ration was significant at P=.05, but the effect of vitamin B_{12} was not significant. This coincides with the findings of Krider *et al.* (1949), Dyer *et al.* (1949), Hill and Branion (1950) and Menge *et al.* (1950).

TABLE 1. VITAMIN B_{12} APF CONCENTRATE AND DRIED WHEY FOR GROWING-FATTENING SWINE

Lot	Treatment[a]	Av. Daily Gain[b]	Av. Daily Feed	Feed per 100 lbs. Gain	No. days to reach Final Wt.
I	Basal ration Gr. yellow corn Exp. soybean oil meal (blended) Vitamin pre-mix No. 2 Minerals incl. trace	1.46	5.44	376.2	115
II	Basal incl. 5 mcg. vit. B_{12} (conc.)[c]	1.60	5.80	366.7	105
III	Basal incl. 10 mcg. vit. B_{12} (conc.)[c]	1.60	6.04	379.5	108
IV	Basal incl. 15 mcg. vit. B_{12} (conc.)[c]	1.53	5.85	385.1	110
V	Basal incl. 15 mcg. vit. B_{12} (cryst.)[c]	1.60	5.80	367.7	108
VI	Basal incl. 2% dried whey	1.62	5.98	377.8	106
VII	Basal incl. 2% dried whey and 15 mcg. vit. B_{12} (conc.)[c]	1.66*	6.20	379.9	103
VIII	Basal incl. 0.44% APF suppl. (Lot 26)	1.73**	6.15	357.1	98
IX	Basal incl. 0.66% APF suppl. (Lot 26)	1.83***	6.51	357.1	94

[a] Ten pigs per lot started 11–17–49 and the following number finished: I-10; II-8; III-10; IV-10; V-10; VI-8; VII-9; VIII-9; IX-10.

[b] Average initial weight 37.43±1.68 pounds per lot.
Average final weight 205.97±2.97 pounds per lot.
Average initial age per lot was 70.2±2.8 days.

[c] Microbiological assay of rations by a modified method of Skeggs *et al.* (1948) and Hutner *et al.* (1948) showed the following vitamin B_{12} values (micrograms per pound): Lot I, 0.36; Lot II, 4.1; Lot III, 8.2; Lot IV, 12.7; and Lot V. 15.4.

* Average daily gains significantly greater than Lot I, P=.05

** Average daily gains significantly greater than Lot I, P≦.01

*** Average daily gains significantly greater than Lot I, P≦.01 and Lot VII, P=.05.

Intermittent scouring occurred in all lots of pigs except the two lots

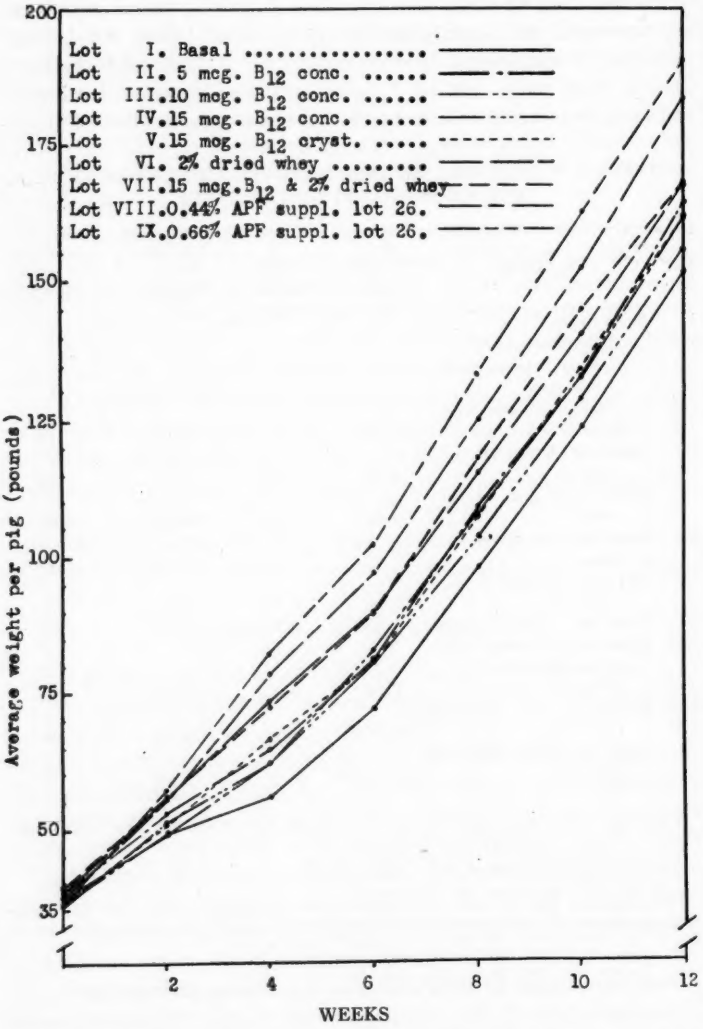

Figure 1. Exp. 473. Growth curves of group mean weights of growing-fattening pigs.

that received the APF supplement. The pigs from lots 8 and 9 also gained considerably faster and required less feed per pound of gain than did the pigs of the other seven lots. This would indicate that the APF supplement supplied factors other than or in addition to vitamin B_{12} and the factor(s) present in dried whey. Since this experiment was completed it has been demonstrated by Burnside *et al.* (1950), Carpenter (1950), Jukes *et al.* (1950) and unpublished work at the Iowa

TABLE 2. BACK FAT MEASUREMENTS

Lot number and treatment	Pig no.	Weight (pounds)	B.f.d.[a] back of shoulders (inches)	B.f.d. middle of back (inches)	B.f.d. middle of loin (inches)	Av. B.f.d. (inches)
I						
(Basal)	3B	223	2.19	1.94	1.72	1.95
	22B	200	1.94	1.67	1.50	1.70
	69B	208	1.94	1.41	1.33	1.56
	130B	200	1.77	1.58	1.25	1.53
	Average[b]	208	1.96	1.65	1.45	1.68
V						
(Basal incl.	2B	238	1.83	1.78	1.75	1.79
15 mcg.	141B	200	1.91	1.91	1.53	1.78
vit. B_{12})	102B	212	2.06	1.56	1.31	1.65
	114B	211	2.02	1.73	1.66	1.80
	Average[b]	215	1.95	1.75	1.56	1.75
IX						
(Basal incl.	30B	233	2.03	1.75	1.56	1.78
0.66% APF	103B	230	2.00	1.66	1.69	1.78
suppl. Lot	140B	210	2.06	1.70	1.34	1.70
26)	160B	204	2.25	1.67	1.64	1.85
	Average[b]	219	2.09	1.70	1.56	1.78

[a] Back fat depth.
[b] No significant differences in average back fat depths between treatments.

Station that it is probably the aureomycin residue of the APF supplement that controls diarrhea, permits more rapid gains and greater feed efficiency.

Growth curves of pigs by lot mean weights are presented in figure 1. The growth data are given for only 12 weeks as several pigs had reached the final weight at this time. The requirement for growth factors not present in the basal ration became apparent after the second week. After the sixth week the pigs apparently had become adjusted to their respective rations and all groups gained at a fairly comparable rate. This indicates that the pigs' need of growth factors was the greatest from the start of the experiment to about 75 pounds. The

growth curves also reveal that there was very little difference in the rate of growth between the lots that received 5, 10 or 15 micrograms of vitamin B_{12} per pound of mixed feed. This would indicate that the vitamin B_{12} requirement of growing-fattening pigs fed in concrete drylot does not exceed 5 micrograms per pound of corn-soybean oil meal ration. The pigs used in this trial were not depleted of vitamin B_{12}. Using semi-depleted pigs Lepley (1950) found the vitamin B_{12} requirement of growing-fattening pigs to be approximately 10 micrograms per pound of corn-soybean oil meal ration.

Table 2, giving the depth of back fat of four barrows from each of the following treatments: basal ration, basal including 15 micrograms of vitamin B_{12}, and basal including 0.66 percent APF supplement, shows that there is a close relationship between body weight and back fat depth. Back fat depth was slightly more in barrows receiving the APF supplement and vitamin B_{12}, but their final weights were greater than those fed the basal ration. Statistical analysis of the data showed there were no significant differences in average back fat depths of the barrows from the three treatments. Apparently the increased growth response obtained by the addition of vitamin B_{12} or APF supplement has little, if any, influence on the depth of back fat.

Summary and Conclusions

Under the conditions of this growing-fattening experiment, using a corn-soybean oil meal basal ration fortified with vitamins and minerals, the following conclusions seem justifiable:

1. Using a vitamin B_{12} concentrate, it appears that the practical vitamin B_{12} requirement of growing-fattening pigs from weaning to 200 pounds in concrete drylot does not exceed 5 micrograms per pound of corn-soybean oil meal ration. A group of pigs fed crystalline vitamin B_{12}, was used as a standard reference.

2. Crystalline vitamin B_{12} or a vitamin B_{12} concentrate increased average daily gains 0.14 pound. This increase approaches significance at $P=0.05$.

3. The addition of 2 percent dried whey and 15 micrograms of vitamin B_{12} per pound of ration significantly ($P=.05$) increased average daily gains by 0.20 pound. Pigs fed rations containing dried whey, with and without vitamin B_{12}, grew at a more rapid rate ($P=.05$), indicating that dried whey supplied growth factor(s) other than, or in addition to, vitamin B_{12}.

4. Highly significant ($P \leqq .01$) increases of 0.27 and 0.37 pound in average daily gains were obtained by the addition of 0.44 and 0.66 percent APF supplement respectively. This APF supplement supplied

factors other than vitamin B_{12} or those present in dried whey, as evidenced by the increased growth rate, improved feed efficiency and absence of scouring in the two groups of pigs receiving the APF supplement.

5. The increased growth response obtained by the addition of crystalline vitamin B_{12}, or the APF supplement had little, if any, influence on the depth of back fat.

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EFFECT OF BODY WEIGHT ON THE BIOLOGICAL VALUE OF PROTEINS FOR GROWING SWINE[1]

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NITROGEN balance, body weight gain, slaughter experiments, and plasma regeneration methods have been employed to evaluate proteins and amino acids fed to various animals. Limitations imposed by facilities and economy often render certain of these impractical for swine investigations, especially when costly dietary ingredients are involved. In this case the nitrogen balance method with its relatively short collection periods may be employed as it permits the estimation of biological values by the Thomas-Mitchell formula (Mitchell, 1924). The biological value of proteins is subject to appreciable variation which limits the usefulness of this method. The level of dietary protein in relation to the requirement of the animal studied is a recognized cause of variation. In recent studies on the methionine requirements of swine (Bell *et al.*, 1950) it became apparent that the biological value of a protein fed at a constant level changed markedly as pigs grew from 10 to 70 kg. in body weight. This paper reports these findings and suggests a method of correcting for these variations.

Experimental

Two groups of four litter-mate weanling Yorkshire barrows were allotted to two replicates of a 4 x 4 Latin square design for study of the effect of methionine supplementation of soybean oil meal. The rations contained about 10% protein (1.68–1.69% nitrogen) supplied by either (a) whole egg, (b) soybean oil meal, (c) soybean oil meal plus egg and methionine, or (d) soybean oil meal and methionine. Combined nitrogen balance and growth studies were conducted and biological values were computed using the method for endogenous nitrogen estimation proposed by Brody *et al.* (1934). Metabolic fecal nitrogen values were determined by Titus' method (1927) (Bell *et al.*, 1950). The metabolic fecal nitrogen is relatively independent of dietary protein, fed at usual levels, and is closely related to the fiber content

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[2] The authors are grateful to Drs. L. A. Maynard and H. H. Williams for advice during the studies and to Dr. C. R. Henderson for reading the manuscript.

of the diet (Mitchell, 1934; Schneider, 1934; and Bosshardt and Barnes, 1946).

Calculation of Endogenous Nitrogen

With the methods now available it is difficult to determine experimentally *valid* endogenous nitrogen values in growing pigs. Nitrogen-free and nitrogen-low diets, if fed for the usual periods, are not compatible with normal growth. Furthermore, there are indications that the minimum endogenous nitrogen excretion, as determined after prolonged nitrogen-free periods, may not be the true value (Allison *et al.*, 1946; Murlin *et al.*, 1946a, 1946b; and Brush *et al.*, 1947). Because of these considerations calculated endogenous nitrogen values were used in deriving the biological values in this report.

Endogenous nitrogen excretion is a function of body weight in homeotherms (Terroine and Sorg-Matter, 1927) and Brody *et al.* (1934) developed a formula for its estimation:

$$\text{Endogenous } N_{mg} = 146\ W_{kg.}^{0.73}$$

The major limitation of this formula is that it applies to *mature* animals and cannot be used to evaluate proteins accurately in growth studies.

Treichler (1939) proposed a formula from rat studies representing the level of endogenous nitrogen excretion during growth and suggested that it could be applied to other species through the medium of Brody's (1945) age equivalence diagrams. The formula was

$$R = 1.2693 + 0.05948\ A - 0.000895\ A^2$$

where R=mg. endogenous nitrogen per basal calorie and A=age (of rats) in weeks.

While this equation has not been tested with growing swine it would seem to have two weaknesses. Firstly there appears to be insufficient data at hand from which to obtain suitable age equivalence involving swine. Growth records reported by Brody and Kibler (1944) and the Mumford data (McKenzie, 1928) as used in Brody's age equivalence diagrams do not represent good growth when compared to growth curves presented by Crampton (1939) and Ellis and Zeller (1939). The latter reported swine attaining the weight of 220 lbs. in about 210 days compared to 360 days for Mumford's pigs which were females involved in a study of the effect of age of breeding upon ultimate size.

Secondly it is difficult to select a formula for the calculation of basal calories. Deighton's (1929) formulas for castrated males,

$$Q/m = 68e^{-.049} + 22 \quad \text{(pigs up to 50 kg.)}$$
$$\text{and } Q/m = 34e^{-.011} + 10 \quad \text{(pigs 50 kg. and over)},$$

where Q is the caloric output daily per kg. in weight, are based on limited observations in the case of pigs under 25 kg., and several of the trials were made under *resting* conditions. Brody and Kibler (1944) reported a formula for *fasting* males $Y_{cal/day}=73.4X_{kg.}^{.80}$. Undoubtedly this formula yields values in excess of basal conditions and it was not derived from pigs growing normally according to present growth standards.

Results and Discussion

The nitrogen balance data and the biological values (Table 1) show clearly that the efficiency of nitrogen utilization for growth decreased

TABLE 1. NITROGEN BALANCE DATA AND BIOLOGICAL VALUES ACCORDING TO WEIGHTS OF PIGS.

Liveweight		No. of observations	Nitrogen			Calculated biological value[1]
Average	Range		Intake	Truly digested	Urinary	
kg.	kg.		gm.	gm.	gm.	
15.07	10–20	8	41.22	36.36	8.11	89.0
24.63	20–30	13	66.99	58.37	12.66	88.4
34.13	30–40	9	92.17	82.48	17.65	87.6
45.20	40–50	6	126.68	118.69	28.38	83.7
53.31	50–60	3	137.47	121.45	32.35	81.8
64.39	60–70	5	139.10	130.80	41.04	77.6

[1] Calculated by using the formula of Brody *et al.* (1934) for endogenous nitrogen estimation, and by assuming 1.8 gm. metabolic fecal nitrogen per kg. dry matter fed.

markedly as the pigs increased in body weight. This decrease is not surprising, since in the Thomas-Mitchell formula a constant dietary nitrogen level, increasing relative to requirements as the pig matures, would lead to increased urinary losses and a lower biological value. As pigs mature the relative amount of protein required for growth decreases and thus, at a constant level of protein intake a decreasing proportion would be used for tissue growth. Furthermore, since the amino acid requirements for growth and maintenance differ, a shift in the use of protein between these functions might affect further the utilization of protein.

The biological values in this study reveal a distinct negative correlation ($r=-0.80$) with liveweight, and with age, since growth was 'normal'. The effect is illustrated in figure 1. The regression line excludes the soybean oil meal diet (b) as it proved to be inferior in biological value.

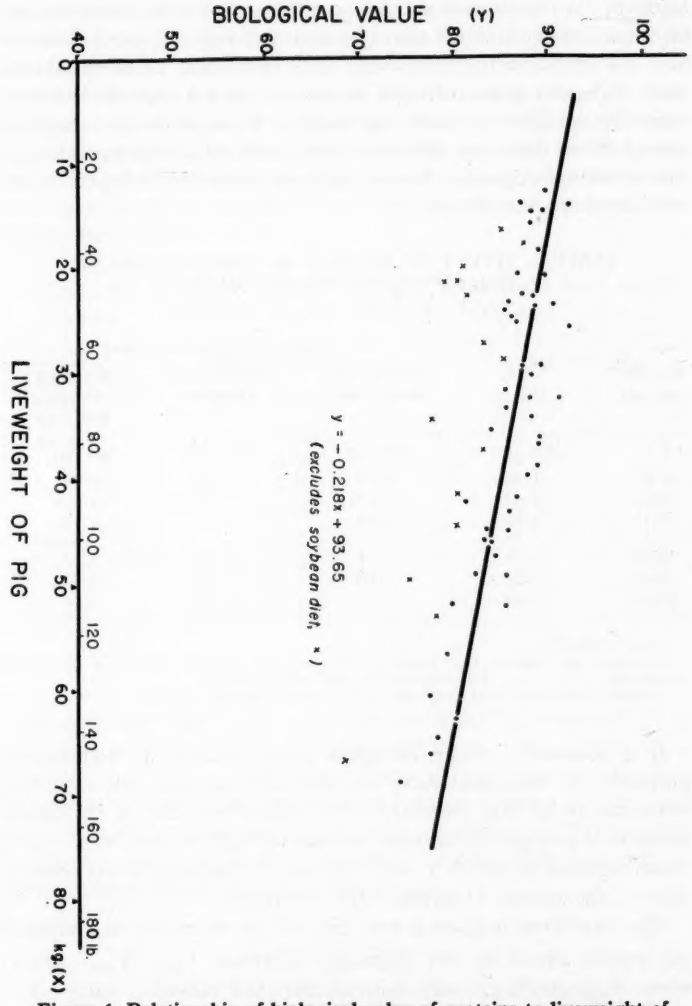

Figure 1. Relationship of biological value of proteins to liveweight of growing swine.

The same general relationships existed whether urinary nitrogen or calculated biological values are studied. In fact the same results were obtained by applying other published values for endogenous nitrogen

(table 2). As further evidence that possible errors in calculating biological values arising from the use of estimated endogenous nitrogen values have not influenced the relationship under discussion, a calculation was made of the endogenous nitrogen necessary to give a constant biological value for the different sized pigs studied. These values are shown in table 2. From these data it is clear that published endogenous nitrogen values fail to compensate for the observed decline in biological values with increasing live weight.

TABLE 2. EFFECT OF METHOD OF COMPUTATION ON ENDOGENOUS NITROGEN VALUES

Average wt.of.pig	Brody 1937[1]	Endogenous nitrogen values		
		Treichler and Brody 1934[2]	Treichler and Deighton[3]	Necessary to yield B.V.=89
kg.	gm./day	gm./day	gm./day	gm./day
15.07	1.03	1.04	1.28	1.03
24.63	1.47	1.57	1.72	1.56
34.13	1.86	2.07	1.87	2.15
45.20	2.27	2.61	2.24	3.83
53.31	2.56	3.00	2.62	4.75
64.39	2.93	3.51	2.95	6.66

[1] $N_{mg}=146W_{kg}^{0.72}$.
[2] Treichler's rat formula with Brody's (1944) formula $Y_{cal/day}=73.4\times_{kg}{}^{.80}$ for resting metabolism.
[3] Treichler's rat formula with Deighton's (1929) basal metabolism formulas.

It is possible to obtain biological values suitable for comparative purposes, and with *measurable* age-effects, by applying the method of covariance to the data (Snedecor, 1946; and others). Any of the present methods of endogenous nitrogen estimation could be used with experiments designed to permit a valid measure of error and having observations on the animals at several stages of growth.

The data shown in figure 1 were analyzed by covariance and adjusted for weight effects by the regression equation $Y_{adj.}=Y_{obs.}-b(x-\bar{x})$ where $Y_{adj.}$ and $Y_{obs.}$=*adjusted* and *observed* biological values; b=*regression coefficient;* x and $\bar{x}$=*observed* and *average* weights of pigs respectively. The within-diet regression of biological value on body weight was $-0.107(P<.01)$. Since the average weights of the pigs on the four treatments did not vary significantly (table 3) the adjusted values for diets show little change in contrast to those variables involv-

ing weight, or age differences (periods). Adjusting biological values in this manner relates them to the average test weight; a factor of special significance when comparing data from various experiments.

Admittedly this procedure fails to yield an exact biological value, which must await improved methods of estimating endogenous nitrogen in growing animals. It does however provide a means for measuring the collective errors introduced by variables associated with growth and development and permits comparisons of biological values without confounding with age effects.

TABLE 3. BIOLOGICAL VALUES OF PROTEINS BEFORE AND AFTER ADJUSTMENT FOR WEIGHT EFFECTS

Diet or collection period	Average wt. of pig	Biological values	
	kg.	Observed	Adjusted
Diet a	34.8	86.0	86.0
b	35.0	79.8	79.8
c	35.0	86.6	86.6
d	34.7	85.6	85.8
Period 1	18.9	87.6	83.6
2	27.6	87.2	84.8
3	39.9	84.5	85.7
4	53.2	79.7	84.0

Summary and Conclusions

Data are presented to show that the biological value of protein fed at a level of 10% decreases as pigs increase in body weight. It is suggested that studies on biological value of protein for growing pigs be designed to permit analyses by covariance as a means of measuring the collective errors introduced by variables associated with advancing weight and age. Comparisons of data from various studies may then be made without confounding with these factors.

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THE EFFECT OF METHIONINE AND CYSTINE ON THE GROWTH OF WEANLING PIGS[1]

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STUDIES on amino acid requirements of weanling pigs have been concerned primarily with the amino acids, tryptophan (Beeson, *et al.* 1949; Shelton, *et al.* 1950a) and lysine (Mertz, *et al.* 1949; Shelton, *et al.* 1950b; Brinegar, *et al.* 1949; 1950). Both of these amino acids are low in corn for growing pigs. In the Corn Belt, soybean oil meal is commonly used as protein supplement to corn to increase both protein quality and quantity. It is now generally recognized that the methionine content of soybean oil meal is low, but whether the combination of corn and soybean oil meal will furnish an adequate amount of methionine for the growing pig is not known since the methionine requirement of the pig likewise is not known.

Bell, *et al.* (1950) have reported results with pigs in which the protein content was approximately 10%. At this level of protein, soybean oil meal was deficient in methionine for the growing pig. Thus, methionine has been proved to be a dietary requirement for the pig. The authors also suggest that on a diet containing 10% protein, the methionine requirement for growing pigs is between 0.07 and 0.27% of the diet. No mention was made regarding the cystine content of the ration. According to Almquist (1947), the methionine requirements of the chick for optimal gain is approximately 0.5% of the diet in the presence of adequate cystine; in the absence of cystine the methionine requirement increases to 0.9% of the diet. Likewise, Womack and Rose (1941) reported that the methionine requirement of the young white rat is 0.5% of the diet when adequate cystine is present, and 0.6% in the absence of cystine.

In the present work, a low-methionine diet containing oxidized casein (Toennies, 1942 and Wilkening, *et al.* 1947) and gelatin plus trypto-

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phan were used as the protein source to study the methionine requirement of the weanling pig.

Experimental Procedure

The basal ration used in this experiment is shown in table 1. It contains 21.0% protein, 0.1% methionine[4] and 0.01% cystine.[4] Lot I received only the basal ration; lot II received the basal ration plus 0.6% L-cystine; lot III received the basal ration, plus 0.2% DL-

TABLE 1. COMPOSITION OF BASAL RATION

Component	%	Vitamin Supplements	
		1. Water soluble vitamins	Mg. daily per kg. live wt.
Oxidized casein	12.0	Thiamine hydrochloride	0.52
Gelatin	10.0	Riboflavin	0.12
Dextrose	39.84	Nicotinic acid	1.20
Dextrin	25.0	Pyridoxine hydrochloride	0.20
Mineral mixture[1]	5.2	Calcium pantothenate	0.50
Lard	5.0	Choline chloride	10.00
Celluflour	2.0		
Liver extract	0.5	2. Fat-soluble vitamins	Levels daily per pig
APF supplement[2]	0.16		
DL-tryptophan	0.3	Vitamin A[3]	3000 I. U.
		Vitamin D[3]	400 I. U.
	100.00	Alpha tocopherol	50 mg.
		Menadione	2 mg.

[1] Heinemann, *et al.* (1946).
[2] Merck's Animal Protein Factor supplement containing 12.5 mg. B_{12} activity per pound.
[3] Fed as Nopco XX.

methionine and 0.3% L-cystine; lot IV received the basal ration plus 0.5% DL-methionine and lot V received the basal ration plus 0.5% DL-methionine and 0.6% L-cystine. Whenever methionine and cystine were added to the ration, they replaced an equivalent amount of dextrose. As a result of the substitutions the rations of lots I, II, III, IV and V contained 0.1, 0.1, 0.6, 0.3 and 0.6% methionine and 0.01, 0.61, 0.01, 0.31 and 0.61% L-cystine respectively.

Ten purebred Duroc female weanling pigs averaging 42.6 lb. were divided equally into the five lots mentioned above. All animals were placed in individual pens equipped with raised wire screen floors and were fed twice daily in amounts to satisfy the individual appetite of

[4] Determined in this laboratory by microbiological assay Dunn (1945). *Lactobacillus fermenti* was used for the methionine assay and *Leuconostoc mesenteroides* for the cystine assay.

each animal. The vitamin supplements (table 1) were mixed with dextrin as a carrier, weighed in such amounts as to furnish the daily requirement of the pig, and fed with each evening feed.

Four days were allowed as an adjustment period so that the pigs on the natural grain ration could gradually adjust themselves to the purified ration. This adjustment period has proved to be extremely desirable when changing animals to a purified type ration especially on experiments of short duration. By allowing this adjustment, the pigs continue to eat normally and the intestinal flora have a chance for adaptation to the purified ration, thereby preventing sudden digestion and absorption disorders which may alter the normal response of the animals. If the cost and type of the purified ration permit, a seven day period is the most satisfactory adjustment period.

Results and Discussion

Even though extreme care is used in selection of thrifty and healthy pigs for experiments of this type, occasionally a pig selected fails to consume the ration and therefore, fails to respond to the diet fed. This occurred in lot V of this experiment. One of the animals in this lot failed to consume the ration and as a result failed to respond normally. It was necessary, therefore, to remove the animal from the experiment at the end of the first two weeks. Observations on the animal were continued for the remaining two weeks of the experiment and it failed to consume a good natural grain ration properly and gained only 6 lbs. during the two weeks on the diet. The other animal on the same ration made very good gains and was normal in all visible respects. Final results have indicated that the levels of methionine and cystine in the ration of lot V were twice the amounts necessary to give optimum growth rate and feed efficiency.

The results of the effect of methionine and cystine on growth rate, feed consumption and feed efficiency are summarized in table 2 and figures 1 and 2. Lot I which received only the basal ration gained at the very low rate of 0.1 lb. per day. These animals were very unthrifty. Figure 1 shows the rate of gain. Actually the animals lost weight during the first two weeks of the experiment. Figure 2 shows the poor feed consumption throughout the 28 days of the experiment. Lot II received the basal ration plus 0.6% L-cystine and made a slight improvement over lot I in average daily gain (0.35 *vs.* 0.1 lb. per day). This increase in gain, with no increase in feed consumption, can be accounted for by the fact that the basal ration actually contained 0.1% methionine, part

TABLE 2. EFFECT OF DIFFERENT LEVELS OF METHIONINE AND CYSTINE ON GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY[1]

Lot number	I		II		III		IV		V
Methionine content	0.1%		0.1%		0.6%		0.3%		0.6%
Cystine content	0.01%		0.61%		0.01%		0.31%		0.61%
Pig number	1	2	3	4	5	6	7	8	9
Initial wt. (lb.)	45.0	45.5	41.5	42.0	41.0	45.5	42.0	41.5	41.5
Final wt. (lb.)	49.0	47.0	50.0	53.5	79.5	78.0	77.0	78.5	78.5
Daily gain (lb.)	0.14	0.05	0.30	0.41	1.38	1.16	1.25	1.32	1.32
Daily feed consumption (lb.)	1.77	1.83	1.81	1.68	3.87	3.66	3.82	3.66	3.54
Feed per pound of gain (lb.)	12.38	34.17	5.97	4.09	2.81	3.15	3.06	2.77	2.68

[1] Duration of experiment was 28 days.

of which was probably being used to form cystine in the animal's body for tissue repair and synthesis of new protein tissue. When an adequate amount of cystine was included in the ration, methionine no longer was converted to cystine and instead could be used as methionine to form new tissue and carry out the other functions for which methionine is

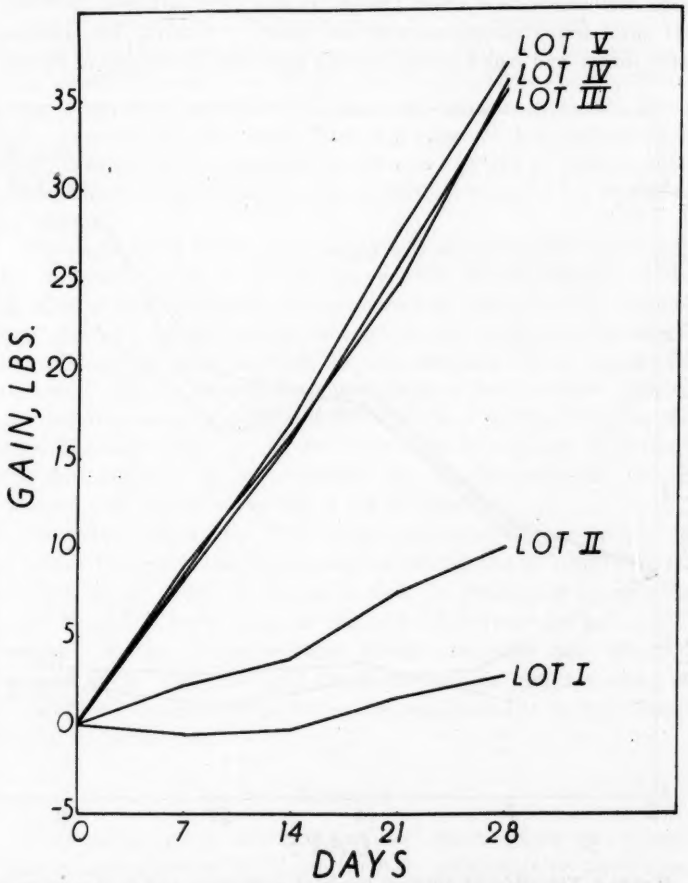

Figure 1. Growth curves of pigs receiving different levels of methionine and cystine. Lot I basal ration (0.1% methionine and 0.01% cystine); Lot II basal ration plus 0.6% L-cystine; Lot III basal ration plus 0.2% DL-methionine and 0.3% L-cystine; Lot IV basal ration plus 0.5% DL-methionine and Lot V basal ration plus 0.5% DL-methionine and 0.6% L-cystine.

required. Thus, more methionine is available as a result of the sparing action of the cystine. This in no way infers that cystine is required as a dietary amino acid, but rather points out the role that methionine plays as the precursor of cystine in the animal body.

Lot III received the basal ration plus 0.5% DL-methionine (total of 0.6% methionine) and a very marked increase in growth rate (1.27 lbs. per day), feed consumption and feed efficiency resulted. The animals were thrifty and had a glossy, smooth hair coat. Reference to figures

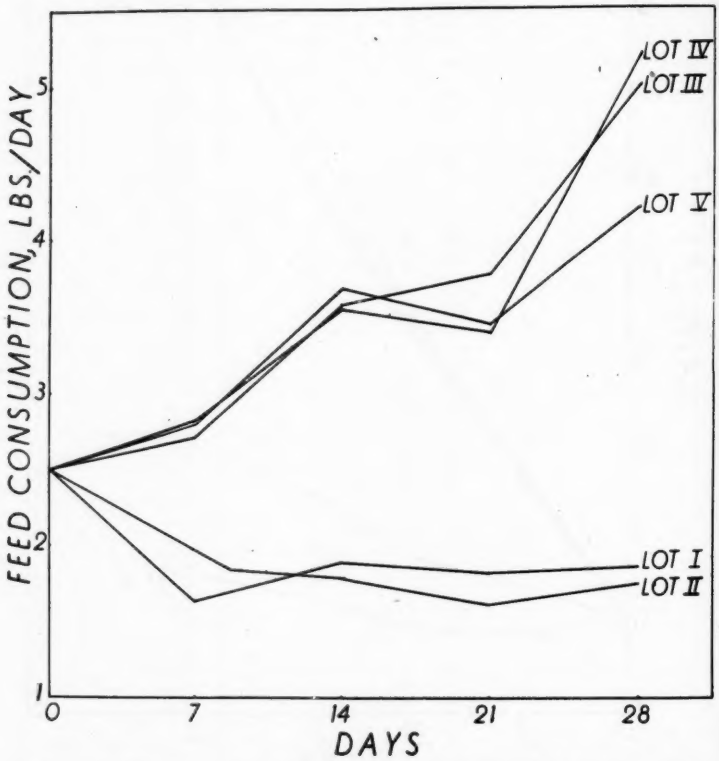

Figure 2. The effect of different levels of methionine and cystine on feed consumption. Lot I basal ration (0.1% methionine and 0.01% cystine); Lot II basal ration plus 0.6% L-cystine; Lot III basal ration plus 0.2% DL-methionine and 0.3% L-cystine; Lot IV basal ration plus 0.5% DL-methionine and Lot V basal ration plus 0.5% DL-methionine and 0.6% L-cystine.

1 and 2 reveals the rapid growth rate and feed consumption of this lot of animals. Although lot V received 0.5% DL-methionine and, in addition, 0.6% cystine, the weight gains, feed consumption and feed efficiency were not significantly higher than the values for lot III. Thus, 0.6% methionine in a ration containing only 0.01% cystine is evidently adequate to meet the needs of the weanling pig.

Lot IV received the basal ration plus 0.2% DL-methionine and 0.3% L-cystine and had essentially the same rate of gain (1.28 lbs. per day), feed consumption and feed efficiency as lot III. There were no differences which could be observed between any animals of lots III, IV or V at the end of the experiment. Thus, it is apparent that levels of 0.3% methionine and 0.3% cystine in the ration are equally as good as 0.6% methionine and 0.01% cystine, or 0.6% methionine and 0.6% cystine in the ration.

The requirement of the weanling pig is thus considerably lower than for the chick. Almquist (1947) has reported the requirement of the chick to be 0.9% methionine in the absence of cystine or 0.5% methionine plus 0.4% cystine. In the chick, about 45% of the methionine can be replaced by cystine. Likewise, Womack and Rose (1941) found that the young white rat requires 0.5% methionine in the diet in the presence of adequate cystine and 0.6% in the absence of cystine. Thus, in the rat 1/6 (about 17%) of the methionine can be replaced by cystine. Our data indicate that approximately 50% of the methionine can be replaced with cystine in the diet of the weanling pig.

On a ration containing 21.0% protein plus adequate cystine (0.3% or more) the methionine requirement of the pig can be tentatively set at 0.3% of the ration. It is possible that the methionine requirement could be slightly lower, since we had no levels between 0.1 and 0.3%; however, in view of the excellent growth rates and feed efficiency attained at the 0.3% level and the extremely poor results attained at the 0.1% level, one would not expect the requirement to be much lower than 0.3% of the diet.

Summary

When purified diets containing oxidized casein, gelatin and tryptophan to make a total of 21.0% protein in the ration were fed *ad libitum*, a methionine deficiency was observed in weanling Duroc pigs. The level of methionine which supported the best rate of gain and feed efficiency was 0.6% of the diet when 0.01% cystine was present in the ration. If adequate cystine was present in the ration, 0.3% methionine sup-

ported a rate of gain and feed efficiency which was equivalent to that obtained when 0.6% methionine plus 0.6% cystine were fed. Tentatively, the methionine requirement for weanling pigs is set at 0.6% of the ration in the absence and 0.3% methionine in the presence of adequate (0.3% or more) cystine.

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PYRIDOXINE DEFICIENCY IN BABY PIGS[1]

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In 1938, Chick and co-workers observed that the pig required certain water-soluble vitamins found in the filtrate and eluate fractions of liver. In the absence of the eluate fraction the pig showed slow growth, epileptiform seizures, and microcytic hypochromic anemias. Upon providing the eluate fraction, the seizures ceased and the blood returned to normal. Hughes (1938), using a pig diet deficient in pantothenic acid and pyridoxine, noted increased gains and improved general physical well-being when a rice bran filtrate was added to the diet.

Wintrobe (1939) found that suckling pigs required certain growth factors found in yeast. When yeast was removed from the experimental diet, the pigs developed convulsions. Wintrobe (1942) observed pyridoxine deficiency symptoms similar to those described by Chick and co-workers (1938). These symptoms were alleviated by the feeding of pyridoxine. Hughes and Squibb (1942) also demonstrated that the pig requires pyridoxine.

It is a known fact that each year large numbers of pigs die between farrowing and weaning. Some of these losses are undoubtedly due directly or indirectly to improper nutrition. With these possibilities in mind, a study of vitamin deficiencies in young pigs was undertaken at this station. The study was designed to compare various deficiencies obtained in the laboratory with those that occur under farm conditions.

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[3] From a thesis to be submitted by W. P. Lehrer, Jr., in partial fulfillment of the requirements for the Ph.D. degree, The State College of Washington.

[4] Experimental work done at the University of Idaho, Moscow.

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This paper reports studies showing pyridoxine deficiencies in baby pigs fed a purified synthetic diet.

Experimental Procedure

The studies reported here consisted of two trials. Three Chester White pigs were used in Trial I and four Duroc-Poland China crossbred pigs in Trial II. In both trials the littermate baby pigs received colostrum and were placed on the experiments at 2 days of age. The experimental animals were housed in individual metal metabolism cages

TABLE I. COMPOSITION OF RATION[1]

Components	Percent	Vitamin added[3]	Mg. per kg. liquid milk
Casein (Labco)	30.0	Thiamine	0.65
Cerelose	37.4	Riboflavin	0.65
Lard	26.6	Nicotinic Acid	2.50
Salts[2]	6.0	Inositol	26.00
		Choline	260.00
		p-Aminobenzoic Acid	2.60
		Pteroylglutamic Acid	0.052
		Biotin	0.01
		Calcium Pantothenate	1.30
		Alpha-tocopherol	1.00
		2-methyl-1, 4-naphthoquinone	0.28
		Vitamin A	2,000 I.U./kg
		Vitamin D	200 I.U./kg

[1] The lard was homogenized with the solution of casein, cerelose and salt to produce an emulsion containing 13 percent solids.
[2] Modified salt mixture of Phillips and Hart (1935).
[3] Control animal also received 0.65 mg. pyridoxine per kg. diet.

designed to allow the separation of urine and feces. The experimental diet was a "synthetic milk ration" (table 1) similar to that described by Wiese *et al.* (1947) for calf nutrition studies, with modification for suckling pigs as reported by Lehrer *et al.* (1949). In these trials, the synthetic milk ration was deficient in pyridoxine, whereas the control animal received the ration plus 0.65 mg. pyridoxine per Kg synthetic milk.

The animals on these trials were fed 7 times each 24 hour day during the early stages of the experiments and 5 times a day during the latter stages. The suckling pig readily learns to drink from a trough, but

TABLE 2. INTAKE AND URINARY EXCRETION OF PYRIDOXINE*

Age in Days	TRIAL I						TRIAL II							
	Normal		Pig 11		Pig 12		Pig 15		Pig 16		Pig 17		Pig 18	
	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day	Intake ug/day	Excretion ug/day
2	0	3	0	7	0	5	0	4	0	4	0	3	0	3
4	273	4					0	2	0	2	0	2	0	4
9			0	34	0	18								
12	546	22					0	5	0	3	0	2	0	8
13	546	35					0	4	0	4	0	4	0	4
15													5000	
16			0	7	0	13								
17									5000					
21			0	4	0	10								
22	780	44					0	3	0	14	0	2	0	5
24	1024	57					0	2	0	9	0	14	0	10
26							0				2000			
28	1267	149					5000	1036	0	38	0	150	0	11
29			10,000	400	0	7	0	9	0	1	0	10	0	3
36	1755	154											200	
37									1000					
39			5,000	400	5,000	400								
40											400			
44	2223	158					0	10			0	10	0	9
46													100	
47			0	29	0	75								
49							0	8				8	0	16
50							40				20		10	
51	2925	97					40	9			20	6	10	2
52							40	8			20	8	10	1
53	3315	267					40	8			20	5	10	4
54							40				20		10	
55	3900	275					40	9			20	15	10	13
56			0	28	0	16								

* Yeast microbiological assay with Sacchromyces carlsbergensis. Modification of the procedure of Atkins, Schultz, Williams and Frey (1943).

some waste is encountered. Because of this, nursing bottles were used in order to keep accurate records of the volume of ration fed.

Urine samples, collected at various intervals during the trials (table 2), were analyzed for pyridoxine.

Results and Discussion

Trial I

During the first 17 days on experiment, pig 11 showed only subnormal appetite and resultant slow growth. On the 17th day the pig vomited, exhibited trembling, showed incoordination of the legs and a tendency to run backwards. These symptoms gradually became more pronounced until the 21st day when the animal had a mild epileptiform seizure. The seizure was quite typical. The pig trembled, was unable to stand, and rolled over on its side or back with legs thrashing wildly. Although weak, the animal was able to stand normally within 3 to 5 minutes after a fit. As the experiment progressed, these seizures occurred more frequently and became more intense. During this period the animal showed poor growth (figure 1) and lack of appetite, developed a rough hair coat, was unthrifty in appearance, was very inactive, and had a dark brown exudate around the eyes. On the 26th day, the epileptiform seizure was very severe and the animal became comatose. Ten mg. of pyridoxine were given on the 28th day, followed by a daily supplementation of 5 mg. for the next 11 days. The pig showed an immediate response to pyridoxine therapy; the appetite improved and growth increased; epiliptiform seizures gradually became less frequent and severe. Five days after pyridoxine supplementation was begun, the seizures stopped and the deficiency symptoms disappeared. The pyridoxine supplement was removed from the ration on the 39th day. The animal did not develop deficiency symptoms again up to the time the expermient was terminated on the 56th day. However, at the end of the trial and removal of the pig from its experimental cage, it was noted the pig ran into objects. Upon close examination it was discovered that there was an impairment of eyesight. Apparently the pig was totally blind since it could not distinguish light from dark.

The other animal in this trial, pig 12, had all the deficiency symptoms exhibited by pig 11, however, these abnormalities were not as severe nor did they occur on the same days. The first epileptic-like fit occurred on the 23rd day. As deficiency symptoms developed, it was noted this animal ate with difficulty. The inability to eat normally appeared to have been due to a loss of the suckling reflex. This and

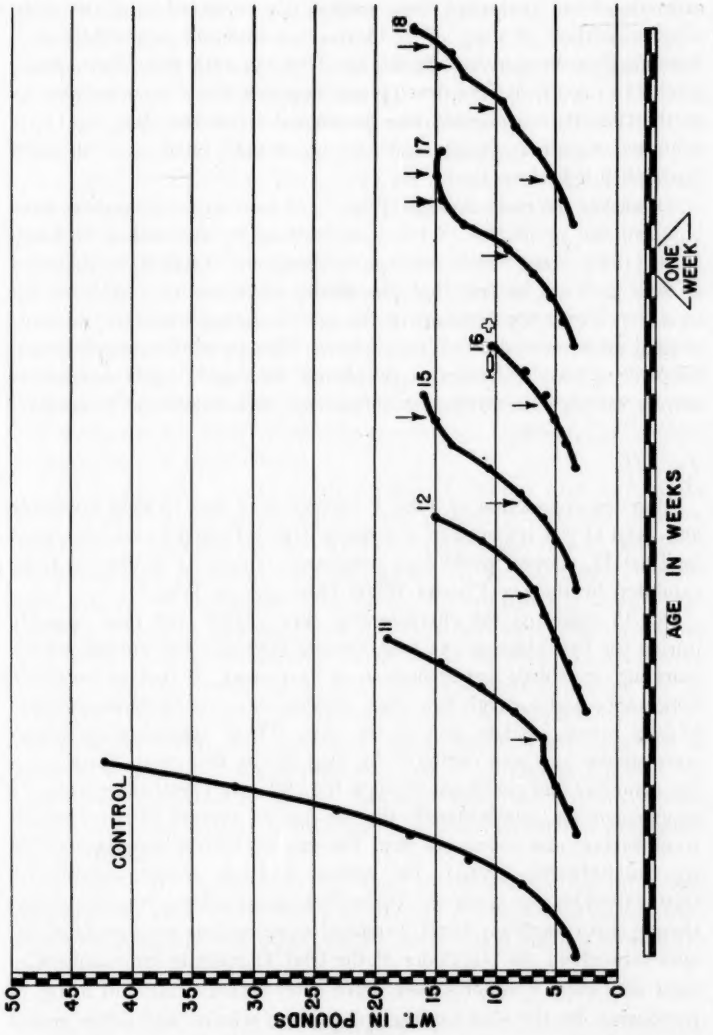

Figure 1. Growth of pigs on pyridoxine deficient diet. The arrows and syringe indicate supplementation of pyridoxine. The cross shows when pig 16 died.

other deficiency symptoms were immediately corrected after the daily supplementation of 5 mg. of pyridoxine was initiated on the 34th day. This supplementation was discontinued on the 45th day. The animal, similar to pig 11, did not develop any new deficiency abnormalities up to the time the experiment was terminated. However, like pig 11, it exhibited impaired eyesight but was not totally blind since it could distinguish light from dark.

At weekly intervals through Trial I, 24-hour urine collections were obtained and pyridoxine excretion determined by the method of Atkin *et al.* (1943) using Saccharomyces carlsbergensis. From data presented in table 2, it can be seen that the urinary excretions of pyridoxine fell to a low level corresponding to the period during which the animals showed the severest deficiency symptoms. After pyridoxine supplementation, the urinary excretion of pyridoxine increased. Again a decrease was shown when the pyridoxine supplement was removed from the diet.

Trial II

After the completion of Trial I, further work was thought advisable and Trial II was undertaken. Crossbred Duroc-Poland China pigs, used in Trial II, showed pyridoxine deficiency symptoms similar to those exhibited by the two Chester White baby pigs in Trial I.

Pig 15 exhibited the characteristic slow growth and poor appetite during the first stage of the trial. On the 16th day this animal started vomiting, trembled, and seemed to be very weak. It had an unthrifty appearance and a rough hair coat, showed excessive lachrymation and a dark brown exudate around the eyes. These deficiency symptoms were similar to those exhibited by pigs in the first trial. Starting on the 22nd day and continued through the 28th day (until the pyridoxine supplement was administered) the pig had an average of two epileptiform seizures and comas per day. The day pyridoxine supplementation was administered (28th), the animal had six severe epileptiform seizures and became comatose. Upon close examination it was established the pig was completely blind. Frequent examinations were made of the eyes throughout the remainder of the trial. Immediate improvement of most deficiency symptoms were noted after administration of 5 mg. of pyridoxine. By the 30th day the epileptiform seizures and comas ceased completely. As the experiment progressed, all deficiency symptoms disappeared with the exception of impaired eyesight. From the 50th day until the termination of the experiment on the 56th, daily supplementations of 40 ug. of pyridoxine were given (table 2). Throughout

this period careful eye examinations were made, but no improvement in eye sight was observed.

Pig 16 showed loss of appetite on the 12th day and was given 5 mg. of pyridoxine on the 17th day. Although it started to vomit on the 36th day, this pig exhibited no epileptiform seizures until the 37th day. However, it had the other characteristic symptoms as described for pigs 11, 12 and 15. Following this seizure the pig was moribund. Although an intramuscular injection of 1 mg. pyridoxine was administered, the pig died a few hours later.

Similar pyridoxine deficiency symptoms were observed in pig 17. This animal exhibited epileptiform seizures from the 22nd through the 25th day. Close examination revealed impaired eyesight. Pyridoxine supplementation was started on the 26th day and continued periodically until the termination of the experiment (table 2). Complete absence of seizures, rough hair coat, poor appetite, brown eye exudate and weakness was noted after supplementation. However, the eyesight impairment was not corrected.

Loss of appetite was observed by pig 18 on the 14th day of the experiment. Five mg. of pyridoxine were administered on the 15th day and then discontinued. As the experiment progressed, typical deficiency symptoms were observed. Vomiting started on the 36th day at which time 200 ug. of pyridoxine were given. An epileptiform seizure and complete blindness were noted on the 45th day. Epileptiform seizures were observed daily thereafter until the end of the trial (58 days) even though 10 ug. of pyridoxine were administered daily beginning on the 50th day. Impaired eyesight was not improved by supplementation.

Urinary excretion and pyridoxine supplementation are presented in table 2. As in Trial I, the urinary excretion of pyridoxine fell to a low level during the period of severest deficiency symptoms. Upon adequate supplementation, symptoms were alleviated and excretion of pyridoxine was increased.

In both trials the excretion of xanthurenic acid in the urine was detected. The growth and thrifty appearance of the control pigs in each trial were comparable to others on the same ration (Lehrer *et al.* 1949 and Wiese *et al.*, in press).

Summary

In these experiments pyridoxine deficiency in baby pigs was characterized by poor appetite, incoordination of the muscles, spastic gait,

poor growth, epileptiform fits, comas, rough hair coat, brown exudate around the eyes, and impairment of eyesight.

The adequate supplementation of pyridoxine cured all the symptoms with the exception of the impairment of eyesight.

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QUANTITATIVE DL-TRYPTOPHAN REQUIREMENT OF THE WEANLING PIG[1]

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BEESON *et al.* (1948, 1949) using a tryptophan-deficient purified ration presented evidence that tryptophan was a dietary essential for the weanling pig. In these experiments 0.4% DL-tryptophan was used in the control diet, and this level gave good growth. Inasmuch as corn and other cereal grains, which are low in tryptophan, constitute the major portion of the pig ration, it seems of practical as well as of fundamental importance to determine the quantitative requirement for this amino acid.

No studies have been reported on the quantitative requirements for tryptophan in swine. Experiments have been conducted to determine the amount of tryptophan required by the chick and young white rat. Rose (1937) has tentatively set the minimum requirement of L-tryptophan for the young white rat at 0.2% of the diet. Almquist (1947) has estimated that the chick requires 0.25% L-tryptophan in the diet; however, Wilkening *et al.* (1947) found the tryptophan requirement for the chick to be as low as 0.18% L-tryptophan and also found that there was no significant difference between a diet containing 0.18% L-tryptophan and one containing 0.30% DL-tryptophan. Lower levels of the DL-isomer were not tested. Later, Wilkening and Schweigert (1947) reported that 17 to 40% of the D-isomer of tryptophan was utilized by the chick. They also reported that 0.30% DL-tryptophan gave the best rate of gain and feed efficiency when fed as the only source of tryptophan in the diet.

The present studies were undertaken to determine the minimum percent of DL-tryptophan that must be added to a tryptophan-deficient diet in order to obtain maximum growth in the weanling pig.

Experimental

Ten purebred Duroc weanling pigs averaging 31 lb. were divided into five lots consisting of two pigs each, a male and a female. These

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[2] The authors are grateful to Dr. J. Waddell, E. I. duPont de Nemours and Co., New Brunswick, New Jersey, for the DL-lysine HCl, and to Mr. A. Lee Caldwell, Eli Lilly & Co., Indianapolis, Ind., for the liver extract used in these experiments.

pigs were closely related, but were not litter mates. All animals were placed in individual pens equipped with raised wire screen floors and were fed twice daily in amounts to satisfy the individual appetite of each animal. An adjustment period of four days was included so that the pigs could make the change gradually from the natural grain ration to the experimental rations.

The basal ration used in these experiments is shown in table 1. It contained 24.5% protein and less than 0.01% tryptophan[3] and was fed to the pigs in Lot I. Lots II, III, IV and V received the basal ration plus 0.1, 0.2, 0.3 and 0.4% DL-tryptophan, respectively, replacing an equivalent amount of dextrose.

TABLE 1. COMPOSITION OF BASAL RATION

Component	%	Vitamin Supplements	
			mg. daily/kg. live wt.
Zein	16.0	Water soluble vitamins	
Gelatin	10.0	Thiamine hydrochloride	0.52
Dextrose	34.3	Riboflavin	0.12
Dextrin	25.0	Nicotinic acid	1.20
Mineral Mixture[1]	5.2	Pyridoxine hydrochloride	0.20
Lard	5.0	Calcium pantothenate	0.50
Celluflour	2.0	Choline chloride	10.00
Liver extract	0.5		
DL-methionine	0.5	Fat-soluble vitamins	Amt. daily/pig
DL-lysine : HCl	1.3	Vitamin A[2]	3000 I.U.
L-histidine : HCl	0.2	Vitamin D[2]	400 I.U.
		Alpha tocopherol	50 mg.
	100.0	Menadione	2 mg.

[1] Heinemann *et al.* (1946).
[2] Fed as Nopco XX.

Purified vitamins were mixed with dextrin as a carrier. An amount of the mixture, to supply the daily requirement of the pig, was weighed and fed with each evening feed. The remainder of the vitamin preparation was stored in an amber colored bottle in a refrigerator.

Nicotinic acid was fed at the rate of 1.2 mg. per kg. of body weight. At the start of the experiment all animals were fed 16 mg. of nicotinic acid per day, but this level was increased as the animals grew. The National Research Council (1944) recommended 7.0 mg. per day for a pig up to 100 lbs. in weight. Therefore, more than twice the recommended level of nicotinic acid was fed. This relatively high level was

[3] Determined in this laboratory by microbiological analysis (Dunn, 1945).

employed in order to minimize the conversion of tryptophan to nicotinic acid *in vivo* (Hundley, 1947; Powick *et al.*, 1948).

Results and Discussion

It can be seen from figure 1 that the amount of tryptophan in the diet had a definite effect on the growth rate of the pigs. Also, it is apparent

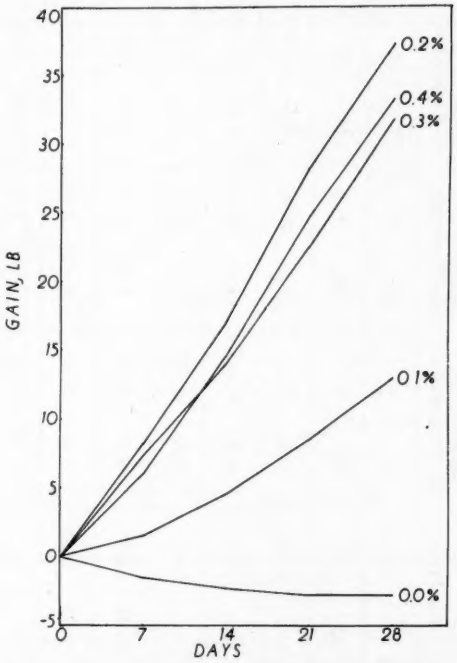

Figure 1. Growth curves of pigs receiving different levels of DL-tryptophan.

that additional DL-tryptophan above 0.2% did not affect the growth rate significantly.

The pigs receiving less than 0.1% DL-tryptophan in the diet lost weight during the 28 days on the experiment (table 2). In addition, they had a remarkably poor feed consumption as compared to the other lots of animals (figure 2). These tryptophan-deficient pigs resembled the

TABLE 2. RESPONSE OF PIGS TO DIFFERENT LEVELS OF DL-TRYPTOPHAN[1]

Item	I Basal Diet		II Basal+0.1% DL-Tryptophan		III Basal+0.2% DL-Tryptophan		IV Basal+0.3% DL-Tryptophan		V Basal+0.4% DL-Tryptophan	
Pig number	1	2	3	4	5	6	7	8	9	10
Sex	M	F	M	F	M	F	M	F	M	F
Initial weight, lb.	34.0	34.5	29.0	32.0	29.0	28.5	29.0	27.5	34.5	34.0
Final weight, lb.	31.0	32.0	40.0	47.0	64.5	67.5	68.5	51.5	65.0	70.0
Total weight change, lb.	—3.0	—2.5	11.0	15.0	35.5	39.0	39.5	24.0	30.5	36.0
Daily weight change, lb.	—0.11	—0.09	0.39	0.54	1.27	1.39	1.41	0.86	1.09	1.29
Daily feed consumption, lb.	0.75	0.87	1.17	1.97	3.93	3.55	3.67	3.25	3.42	3.79
Feed per pound of gain, lb.	—	—	4.34	3.68	3.10	2.55	2.60	3.79	3.14	2.95

[1] Duration of experiment=28 days.

ones previously described by Beeson *et al.* (1949). The animals which received 0.1% DL-tryptophan made small gains (0.47 lb. per day) during the same 28-day period. They consumed more feed than those receiving no tryptophan, but less than 50% of the amount consumed by the animals receiving 0.2, 0.3, or 0.4% tryptophan (figure 2). Likewise, the feed efficiency for animals receiving 0.1% tryptophan was poorer than for the lots receiving higher levels of tryptophan. Four lb. of feed containing 0.1% DL-tryptophan were required to produce 1 lb. of gain, whereas about 3 lb. of feed were required to produce 1 lb. of

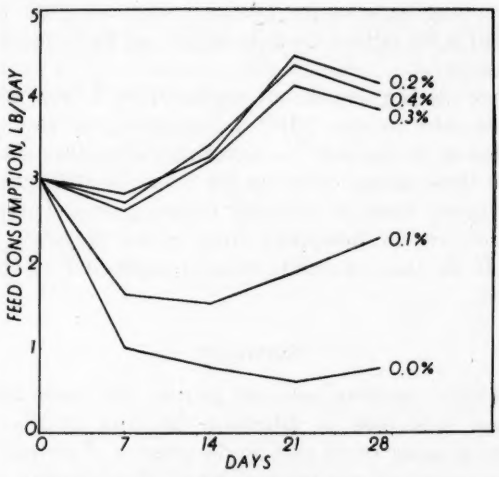

Figure 2. The effect of different levels of DL-tryptophan on feed consumption.

gain at the higher levels of tryptophan. Thus, it is apparent that the lack of a sufficient quantity of tryptophan in the diet reduces markedly the feed efficiency.

The growth rate, feed consumption and feed efficiency for animals receiving 0.2, 0.3, or 0.4% tryptophan were much higher than for animals receiving 0.1% tryptophan. It is obvious that 0.1% DL-tryptophan did not support a growth rate comparable to rations containing higher levels of tryptophan. On the other hand, there are no significant differences in the growth rates of pigs fed rations containing 0.2%, 0.3% and 0.4% DL-tryptophan. These data indicate that a ration containing 0.2% DL-tryptophan supplied sufficient tryptophan

for optimum growth and efficiency of gain in the pig. Maximal rates of gain were obtained with this diet and higher levels of DL-tryptophan did not result in an increased growth response.

Using a protein which differs markedly from that used in a previous experiment (Beeson *et al.*, 1949), it has been possible to confirm the finding that tryptophan is an essential amino acid in the diet of the weanling pig. An accurate comparison cannot be made since the conditions of the two experiments were not identical; nevertheless, the animals receiving a tryptophan-deficient diet lost weight and had poor feed consumption, depraved appetite, rough hair coat and symptoms of inanition in both experiments. Likewise, when adequate tryptophan was provided in the rations, the rates of gain and feed efficiency were in good agreement.

In view of the fact that the rat requires 0.2% L-tryptophan in the diet and the chick requires 0.18% L-tryptophan, the requirement for L-tryptophan in the pig must be considerably lower than that observed in either of these species, unless the pig is able to utilize the D-isomer to a large extent. Since the naturally occurring form of tryptophan is the L-isomer, rations formulated from protein feedstuffs would be adequate on the basis of results reported herein if 0.2% tryptophan were present.

Summary

Purified diets containing zein and gelatin, plus amino acid supplements, have been used to determine the level of DL-tryptophan required by weanling Duroc pigs. In the presence of adequate nicotinic acid maximum growth was obtained when DL-tryptophan constituted 0.2%, or more, of the diet.

Pigs receiving 0.1% DL-tryptophan consumed approximately one-half as much feed, and gained at about one-third the rate of the pigs receiving 0.2% DL-tryptophan. The feed efficiency of the pigs receiving the lower level of DL-tryptophan was reduced.

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PANTOTHENIC ACID DEFICIENCY IN BABY PIGS[1, 6]

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CHICK, Macrae, Martin and Martin (1938) reported that weanling pigs fed a purified ration deficient in the "filtrate factor" lost weight, scoured, showed a weakness in the hind quarters and walked with a swaying gait. This condition was not cured by thiamine, riboflavin or nicotinic acid; yeast and certain filtrate fractions of liver were beneficial. Hughes (1939) observed that weanling pigs fed a purified diet known to be deficient in the filtrate factor developed a "goose-stepping" gait. Wintrobe, *et al.* (1942) and Follis and Wintrobe (1945) reported that pantothenic acid deficiency in the pig resulted in poor growth, alopecia, cough, excessive nasal secretion, diarrhea, spastic gait and jerky "goose-stepping" with the hind legs. These workers also observed ulcerative colitis, necrosis of the intestinal wall and degeneration of the myelin sheath of the brachial and sciatic nerves. The work of Maynard (1947) has shown that dermatitis and a dark brown exudate around the eyes are characteristic symptoms of pantothenic acid deficiency in the pig. Hughes and Ittner (1942) reported that the minimum daily requirement for the pig was approximately 10 mg. of pantothenic acid per 100 pounds of live weight.

In this paper we are reporting studies on pantothenic acid deficiency in baby pigs.

Experimental Procedure

Three male Poland China (trial I) and four Duroc (trial II) pigs, 48 hours old were used as experimental animals. The pigs in each trial

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[6] We wish to acknowledge the assistance of Mrs. June Anderson and Mrs. Sybil Brislain with the vitamin analyses. Credit is due Dr. P. G. Eldredge for assistance with post mortem studies.

We are also indebted to Merck and Company, Rahway, New Jersey, through the courtesy of Dr. D. F. Green, for supplies of thiamine, riboflavin, pyridoxine, nicotinic acid, inositol, choline, p-Aminobenzoic acid, biotin, calcium pantothenate and tocopherol; to Lederle Laboratories Inc., Pearl River, New York, through the courtesy of Dr. E. R. L. Stockstad, for pteroyl-glutamic acid; to E. I. Dupont De Nemours and Company, New Brunswick, New Jersey through the courtesy of Dr. S. Waddell, for crystalline vitamin D_2 and to Wilson and Company, Chicago, Illinois, through the courtesy of Dr. S. W. Hier, for the 1:20 liver powder.

were litter mates. They were kept in individual metal cages 2.5 by 2.5 feet in size, equipped with wire mesh bottoms and underlying screens to facilitate the separation of urine and feces. The experimental diet was a "synthetic milk" having the composition given in table 1 and containing no pantothenic acid. The control animals were fed this ration plus 1.30 mg. calcium pantothenate per kg. "synthetic milk". For complete details concerning the preparation of this diet see Wiese, *et al.* (1947). The feeding, care and diet modifications for baby pigs were the same as that described by Lehrer, *et al.* (1949).

TABLE 1. COMPOSITION OF RATION[1]

Component	Percent	Vitamins Added[3]	Mg. Per Kg. Milk
Casein (Labco)	30.0	Thiamine	0.65
Cerelose	37.4	Riboflavin	0.65
Lard	26.6	Nicotinic Acid	2.50
Salts[2]	6.0	Inositol	26.00
		Choline	260.00
		p-Aminobenzoic Acid	2.60
		Pteroylglutamic Acid	0.052
		Biotin	0.01
		Pyridoxine	0.65
		Alpha-tocopherol	1.00
		2-methyl-1, 4-naphthoquinone	0.28
		Vitamin A	2,000 I.U./kg.
		Vitamin D	200 I.U./kg.

[1] The lard was homogenized with the solution of casein, cerelose and salts to produce an emulsion containing 13 per cent solids.
[2] Modified salt mixture of Phillips and Hart (1935).
[3] Control animals also received 1.30 mg. calcium pantothenate per kg. liquid diet.

Results and Discussion

Trial I

During the first two or three days on the experimental diet the pigs scoured. This was apparently due to over feeding since the condition was alleviated when the food intake was decreased.

Excessive lachrymation, coughing and a decrease in appetite were observed after the animals had been on the experiment for 9 days. By the eleventh day dermatitis was noted on the top of the neck and shoulders. In addition the pigs showed incoordinated movements of the hind legs and a spastic gait.

By the twentieth day the dermatitis was very pronounced on the top of the head, neck, shoulders, over the back and down the hind legs. The hair coat was dull; the movements of the hind legs were incoordi-

nated and the animals "goose-stepped" when walking. A heavy dark brown exudate appeared around the eyes and there was loss of hair from the neck, shoulders, back and rump. The pigs scoured and had an impairment of the sucking reflexes. The animals lost control of the tongue and were able to eat with difficulty. The animals made poor

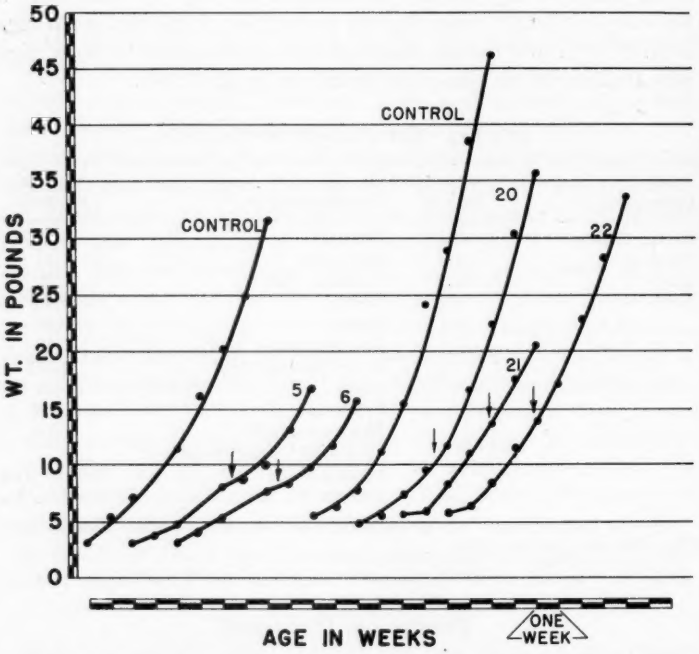

Figure 1. Growth of pigs on pantothenic acid deficient diet. The arrows indicate when feeding of calcium pantothenate began.

gains in weight and, as can be seen from figure 1, were far below the corresponding weight of a litter mate control pig. The photograph of pig 6, figure 2, illustrates the appearance of pigs when deficient in pantothenic acid. The poor hair coat and dermatitis on the legs are evident in the photograph. Figure 3 shows a comparison of a pantothenic acid deficient pig and one of the litter mate control animals.

On the thirty-third day the animals were given one mg. of calcium pantothenate orally; this supplement was continued for the next 18

days. Two days after supplementation was begun, the appetite of the pigs increased; the sucking reflexes returned to normal and the scouring ceased. As can be seen from figure 1 the animals showed an increase in body weight. During this period of supplementation there was no improvement in the appearance of the hair coat; the dermatitis and

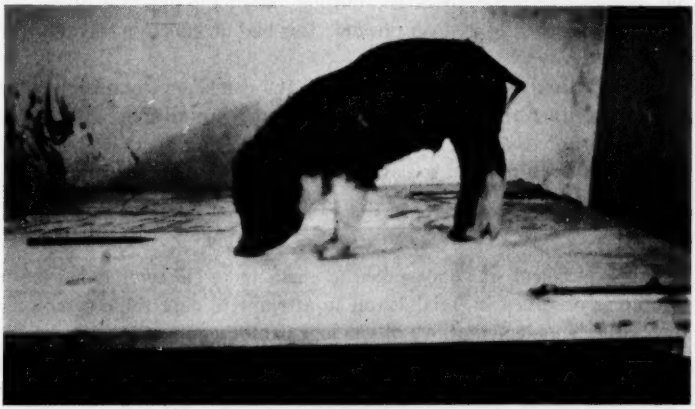

Figure 2. Pig 6 after being on pantothenic acid deficient diet for 30 days. Note poor hair coat and dermatitis on legs.

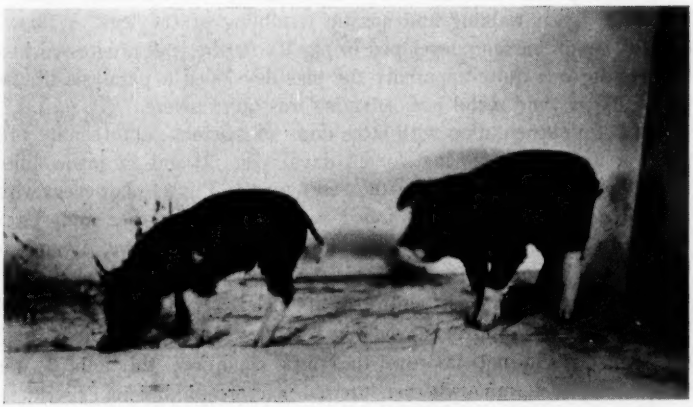

Figure 3. Comparison of pantothenic acid deficient pig and a litter mate control animal. Note appearance of control animal on right.

the dark brown exudate around the eyes still persisted. The animals continued to show incoordinated movements of the hind legs and "goose-stepping".

On the fifty-first day the calcium pantothenate supplement was increased to 10 mg. per day. This did not result in an improvement of the condition of the animals. In addition one of the pigs was given one gram of Wilson's 1 to 20 liver powder; this had no effect on the animal's condition.

The fact that we were unable to cure the deficiency completely may be due to either the low amount of calcium pantothenate given as a supplement, or, the pigs had been so severely depleted that they were no longer able to respond to calcium pantothenate administration.

Trial II

This experiment was undertaken to check more closely the deficiency symptoms observed in Trial I and to attempt to cure these symptoms with larger supplementations of calcium pantothenate.

No scouring was encountered during the early stages of this trial as was the case in the early stages of Trial I. Scouring was prevented by allowing the animals to eat just the quantity they desired.

During the first two weeks of this trial, typical dermatitis, rough, dull hair coat and dark brown eye exudate appeared on all animals. These symptoms were gradually followed by incoordinated "goose-stepping" when walking and spastic trembling of the legs. A "bow-legged" condition soon developed in pig 20. As the trial progressed loss of appetite was quite apparent; the pigs developed a paralysis of the hind quarters and rectal hemorrhaging was quite severe.

Upon supplementation with large doses of calcium pantothenate (10 mg. for 8 days and 20 mg. for 13 days) pigs 20 and 22 made quite rapid recovery and good growth; see figure 1. Pig 21, however, with typical deficiency symptoms failed to respond to smaller and fewer calcium pantothenate supplementations (1 mg. for 2 days, 5 mg. for 6 days and 10 mg. for 1 day). This pig continued to tremble severely, was unable to walk, lost control of its reflexes, could not stand and refused to eat. Rectal hemorrhaging was very profuse. As these small supplements did not improve the pig's condition, the animal was sacrificed to determine whether internal macroscopic lesions existed.

The animals in both trials were sacrificed and postmortem examinations failed to reveal any internal gross lesions. It was observed that the deficient animals had little subcutaneous fat and the internal fat

TABLE 2. URINARY EXCRETION OF PANTOTHENIC ACID[1]

	Trial I						TRIAL II						
	Normal		Deficient				Normal		Deficient				
Age			Pig 5		Pig 6				Pig 20		Pig 21		Pig 22	
In Days	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day	Intake ug/day	Excre. ug/day
2	410	104	0	49	0	92	390	585	0	423	0	296	0	432
3	630	59	0		0	42								
7	770	46	0	80	0	44	700	410	0	90	0	138	0	159
12	1100	58	0	11	0	14								
15							1560	293	0	23	0	50	0	27
21							2030	106	0	40	0	16	0	22
27							3120	90	500	53	0	75	0	151
28							3120	169	200	56	1000	62	1000	43
30							3900	127	10000	110	5000	91	5000	136
33			1000	3	1000	4								
34			1000	33	1000	10								
35			1000	12	1000	19	5460	432	10000	987	10000	148	10000	4
36	2510	420	1000	8	1000	41								
42							6630	277	10000	1356		52	10000	689
48	2990	524	1000	65	1000	12			20000	2493			20000	3216
54	3900	666	10000	188	10000	17			20000	10512				
55													20000	13

[1] Microbiological assay using L arabinosus based on the procedures of Skeggs and Wright (1944).

was lacking. The ribs and long bones of the legs were soft and easily fractured; the bone marrow was abnormally light in color.

During the course of the experiment 24 hour urine collections were made and the pantothenic acid excretion determined by the method of Skeggs and Wright (1944). The data given in table 2 shows that the urinary excretion of pantothenic acid dropped to a very low level by the thirty-third day (Trial I) and twenty-eighth day (Trial II). After the feeding of calcium pantothenate, there was an increase in the excretion of pantothenic acid. This increase, proportional to the intake in Trial I, would indicate that the animals were severely depleted of pantothenic acid—a possible explanation of the inability of the pigs to recover from the deficiency when small amounts of calcium pantothenate were supplied. In Trial II the feeding of 10 to 20 mg. of calcium pantothenate per day resulted in high urinary excretion of pantothenic acid.

The urinary excretion of thiamine, riboflavin, nicotinic acid, N^1-methyl-nicotinamide, pyridoxine, biotin and pteroylglutamic acid by pantothenic acid deficient pigs was also determined. The excretion of these vitamins by the deficient pigs was not different from that of the control animals.

Summary

Pantothenic acid deficiency in the young pig is characterized by poor growth, loss of appetite, scours, lachrymation, dermatitis, coughing, loss of the sucking reflex, a dark brown exudate around the eyes, spastic gait, "goose-stepping", alopecia, and low urinary excretion of pantothenic acid.

The daily feeding of one mg. of calcium pantothenate resulted in an improvement of appetite and growth and cessation of scours. There was no great improvement of the other symptoms. This was probably due to the severe depletion of the animals since they were unable to respond completely to the administration of calcium pantothenate. However, the daily supplementation of 10 to 20 mg. of calcium pantothenate resulted in complete recovery and great improvement of appetite and growth.

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THE DESIGN AND OPERATION OF METABOLISM UNITS FOR NUTRITIONAL STUDIES WITH SWINE

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THE importance and adaptability of swine to nutritional and biological experimentation is demonstrated by the number and variety of studies currently appearing in the literature. In addition to the conventional swine studies, there is at present a great interest in such factors as aureomycin, folic acid and antagonists, vitamin B_{12}, general mineral metabolism, radioisotope studies, and internal and external radiation effects to provide for extrapolation to humans from the standpoint of therapeutic use of isotopes and radiation tolerance. However, the acquisition of detailed data from quantitative and qualitative studies of nutrient requirements has necessitated the use of expensive commercial cages and equipment which have not usually been found satisfactory. The shortcomings of this type of cage for balance studies have been discussed by Dinning, Gallup and Briggs (1948). This situation has resulted in the general acceptance of growth and weight increase as a major criterion of measurement for response to the many nutritional factors. The experimental design has thus been limited in many instances for lack of simple equipment and techniques for a more thorough study of the problems involved.

The recent widespread introduction of radioisotope labeled elements and compounds into the realm of nutritional experimentation has increased the need for the development of improved techniques in conducting balance studies with swine. The separation of urine and feces eliminated must be complete and quantitative, and area contamination from animal excreta minimized. The necessity for this additional requirement of minimizing area and animal contamination in radioactive studies has made revisions in the early cages developed by Forbes (1915) mandatory. In radioisotope studies it is especially important that provisions be made for convenient and quantitative dosage of the animal in the stall, and also for the collection of samples.

It has been necessary therefore to design a metabolism unit for swine with modifications that would fulfill the above conditions and effect

the satisfactory quantitative separation of urine and feces eliminated by gilts, barrows or boars. This has been accomplished by the unique use of a hot wire arrangement from an electric fence unit that serves to restrain the animal without discomfort and without physical contact

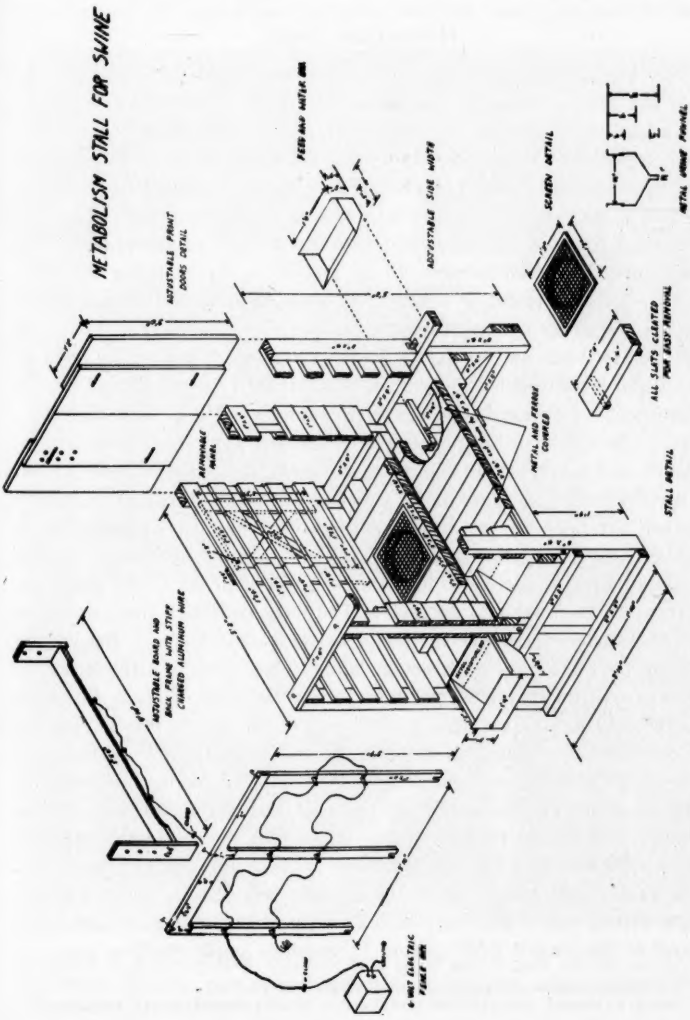
METABOLISM STALL FOR SWINE
ADJUSTABLE FRONT DOORS DETAIL
FEED AND WATER BOX
ADJUSTABLE SIDE BOARD
SCREEN DETAIL
REMOVABLE PANEL
METAL AND FENCE CORDS
ALL SLATS CLEATED FOR EASY REMOVAL
STALL METAL
METAL URINE PANEL
ADJUSTABLE BOARD AND BRACE LINE WITH STEEL CHARGED ALUMINUM WIRE
HOT ELECTRIC FENCE WIRE

Figure 1. A linear perspective of a two unit metabolism stand for swine.

thus permitting the collection of feces with the avoidance of smearing on the restraining surfaces. Urine collection is accomplished by means of an adjustable stand and adaptable urinals for male and female. Devices are described that make it possible to immobilize the animal at will for dosage and blood sample collection.

Metabolism Units

The construction of a two-unit metabolism stand for boars or gilts that has proven adequate for balance studies is illustrated in figure 1. This unit is adaptable, by adjustments, to either males or females weighing 75 to 350 pounds (figure 2). The length may be adjusted by sliding the feces collection pan forward or backward simply by removing or adding the removable 2-in. x 4-in. boards in the floor. The position of the urine funnel is likewise adjustable to the size of the animal. The width may be varied between 12 in. and 20 in. by swinging the front outside panel outward or inward and securing it at the desired width with a pin in the matched holes or by inserting a false side. The combination feed box and waterer is inserted drawer fashion at the front of the stand slightly below floor level to facilitate removal for refilling or cleaning. Its position below the floor minimizes spillage onto the floor. The 12-in. metal urine funnel is attached to two jointed 2-in. x 8-in. boards and covered with No. 9, ¾-in. mesh wire. The two-piece construction facilitates removal for cleaning or decontamination. A rubber funnel[1] strapped to the barrow with a harness and connected to a rubber hose in the usual manner may be readily substituted for the grill and metal funnel when high radioactivity is involved and minimum surface contamination is necessary. The feces collection pans, with the sloping front, are similar in design to those described by Briggs and Gallup (1949) for use with wether lambs. These may be either stainless steel or galvanized metal, depending upon the type of study involved. A layer of heavy paper taped to the inside of the pan facilitates cleaning and minimizes contamination especially when radioactivity is involved. The stall for boars or barrows may be converted readily for use with gilts by removing the feces collection pan and sliding the 2-in. x 8-in. boards holding the funnel out to the rear of the rack and replacing them with similar 2-in. x 8-in. boards without the funnel attachment. The 2-in. x 6-in. boards where the animal's feet rest are covered with a light metal, and coated with ferrox[2] to prevent slipping. A removable panel at the outside front (figure 1) provides ample working space for

[1] Soft rubber funnels—available from Deval Rubber Co., Providence 2, R. I.
[2] Ferrox, an abrasive paint—available from American Abrasive Metal Company, Irvington, N. J.

dosing and routine bleeding from ear or jugular vein without removing the animal from the metabolism stall.

A novel feature of this design is the reinforced restraining unit. Two or three charged wires, adjustable inward across the rear opening of the stall at hock and ham levels, from a battery electric fence unit discourages the animal from stepping into the feces container or from

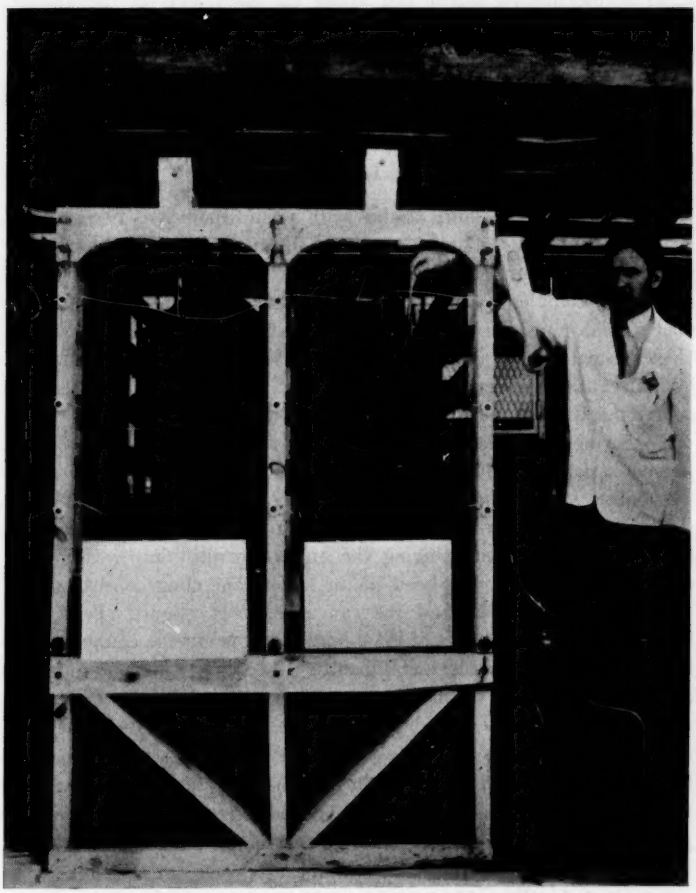

Figure 2. A metabolism unit for male (left) and female (right) swine in operation. The hot wire restraining unit and the sow urine conduit with attachments is demonstrated.

backing out of the stand. These wires are attached to insulators on a removable 2-in. x 2-in. board rack for convenience of operation (figure 1). The unit is grounded to the metal covering the 2-in. x 6-in. boards under the animal's fore and rear feet and to the feces collection pan. Another wire is attached by insulators to an adjustable 1-in. x 4-in. x 5-ft. board, hinged to the front panel and secured to the rear of the stand at the desired height just above the back level of the animal. This discourages him from climbing the sides of the stall. This unit has been used to restrict the animal for periods of three to four weeks without apparent discomfort, undue excitement, hampering of movements, and without interfering with the collection of urine and feces

The Swine Immobilizer Unit

In nutritional or balance studies with swine where it is necessary to dose quantitatively the animal orally or intravenously, or if blood samples are needed during the balance period, it is desirable to immobilize the animal completely without removal from the metabolism stall. This is of special importance when radioactivity is involved since the surface of the animal may be above the radiation tolerance and it is necessary to minimize area and animal contamination by the isotope or excreta containing the radioactivity.

The swine immobilizer unit as designed and operated (figure 3) has proven satisfactory for securing the animal's position in the swine metabolism stand. The unit consists of three parts; (1) the Snare (2) the Sling and (3) the Head Stock. The small chain snare (figure 3A) facilitates holding and bringing the animal forward into position for hoisting and securing the head in the stock. The sling consists of the windlass (figure 3B-1) and the canvas support (figure 3B-2). The windlass is made of wood and is supported on the outside of the stall by means of two removable metal hooks. The heavy canvas support is attached at one end by metal hooks to the side of the stall opposite the windlass. The other end passes under the animal's belly between the rear and fore legs and is fastened to the windlass. The animal can thus be hoisted off its feet and into position for securing the head in the metal head stock (figure 3C). The head is fastened and held in position by passing a small chain through the animal's mouth and hooking it over the opposite side of the nose piece of the stock. The metal stock is adjustable to the variable width of the stall, and both the windlass and stock may be removed readily for use in adjoining stalls. This simple equipment has served satisfactorily for total immobi-

lization of swine weighing 100 to 350 pounds for quantitative administration of radioactive isotopes *per os* or in the ear vein, and for routine bleeding from the jugular or ear vein.

SWINE IMMOBILIZER UNIT
A - THE SNARE
B1 - WINDLASS TO ADJUST SLING
B2 - HEAVY CANVAS OR 3" x 45" BELTING
B - THE SLING
C - THE HEAD STOCK
HEAD STOCK IN POSITION
ADJUSTABLE SIDE MOUTH HELD SECURE BY BOLT OR NAIL
CHAIN

Figure 3. The swine immobilizer unit illustrating construction details and operation position for the three parts (1) the snare (a), (2) the sling (b) and (3) the head stock (c).

Collection of Urine from Gilts

In balance studies involving the necessity for the quantitative separation of urine and feces from gilts or mature sows the urine conduit shown in figures 3 and 4 has proven satisfactory for balance periods of 5 to 7 days. This separator is an adaptation of the urinal for heifers as described by Hobbs, Hansard and Barrick (1950). It is readily constructed from a 36-in. length of a 2½-in. seamless rubber tubing.[3]

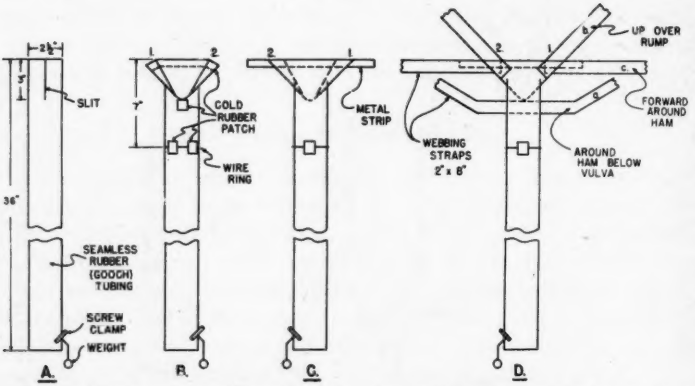

Figure 4. The sow urine conduit showing details of construction, a, b, c and harness for attachments to the rump of the animal d.

A 3-in. incision is made along the side of one end of the tube, and the terminal point reinforced with a cold rubber patch (figure 4A-B). A ½-in. strip of cold rubber patching is applied to reinforce the top outside edge of the conduit (figure 4B). A wire ring is attached by cold rubber patches 4-in. below the terminal point of the incision to the outside of the upper portion of the tube (figure 4B-C). This ring holds the conduit open as a primary receptacle and permits a free flow of the urine. A thin piece of light metal, ½-in. wide and 5-in. long, is attached along the top outside of the tube by means of small screw clamps (figure 5). This metal strip permits adjustment to the contours of the animal and allows for the maintenance of a closer contact of tubing with the skin area between the anus and vulva. If the animal is fat a similar metal strip may be desirable at the lower side of the cup to facilitate adjustment between the hams. The reinforced or cupped end of the urine conduit is placed over the vulva and secured to the gilt's rump by three pairs of 2-in. x 8-in. webbing straps (figures 4D and 5). The straps are attached to the conduit by means of open

[3] "Gooch tubing"—available from the Goodrich Rubber Co., Akron, Ohio.

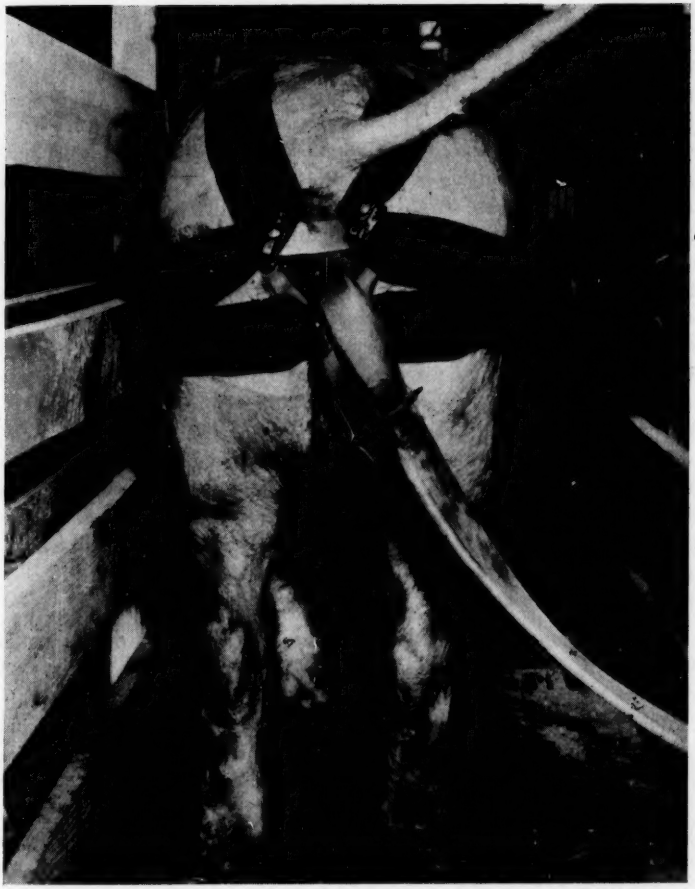

Figure 5. The sow urine conduit in operation showing details for attachment to the rump of the animal for satisfactory separation of urine from feces.

sided 1-in. clamps, and to the animal by means of branding cement.[4] It has been found most convenient to assemble the conduit and make the attachments in the following order: (1) The lower pair of straps (figure 4D-a) is attached to the conduit at the terminal point of the incision in the tube, and extends horizontally around the outside of the

[4] Branding cement—available from the Nebraska Salesbook Co., P. O. Box 548, Lincoln, Neb.

hams just below the vulva to hold the lower side of the cup in position (figure 5). (2) The top pair of straps (figure 4D-b) is attached to the outside top of the cup and extends parallel upward over the rump midway between the hip and backbone for vertical support to the tube (figure 5). (3) The middle pair of straps (figure 4D-c) similarly attached to the conduit, serves to stretch the cup to each side and downward, permitting position adjustment in the area between the anus and vulva. These straps extend horizontally from a point just above the pin bones forward and slightly downward to a point 3-in. to 5-in. below the hips (figure 5). The section of skin between the anus and vulva is cleaned with alcohol, and the conduit between the top clamps is cemented thereto. The metal strap along the top outside of the cup is then adjusted inward near the center to prevent the possibility of any contamination of the urine with fecal material should the seal be broken for any considerable period of time before repair. It has been found desirable to inspect this seal and make adjustments daily. The other end of the conduit is then passed through the opening in the side of the stand outward and downward to a collection carboy where it is maintained in position by a small weight sufficient to cause it to slide back into the bottle when it is shifted by the animal's movements (figure 2).

Summary

A description is presented of the design and operation of adjustable metabolism units which are adaptable for use in balance studies with barrows or gilts. The width of the stall and the length may be readily adjusted to the size of the animal. The animal is successfully restricted to the desired area by means of these adjustments and a hot wire arrangement from an electric fence unit. A drawer-type combination feed box and watering unit attached below floor level minimizes contamination of excreta with feed and water. A removable panel in one side of the stand and a swine immobilizer unit attachment facilitates bleeding or dosing of the animal without the necessity of removing it from the stall.

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SPECIFIC GRAVITY AS A MEASURE OF THE FAT CONTENT OF THE PORK CARCASS[1]

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IN recent years the low price of lard and the price differential between fat and lean cuts have emphasized the need for reliable measures of the fat and lean content of the pork carcass.

Recent work with guinea pigs reveals a close association between the specific gravity and the fat content of the eviscerated carcass (Rathbun and Pace, 1945). Several studies in man have indicated a strong relationship between specific gravity and obesity in the human body (Behnke *et al.*, 1942; Zook, 1929; Boyd, 1933). Warner *et al.* (1934) have ranked the methods of determining the actual fatness in the pork carcass in order of their probable accuracy as follows: chemical analysis of the entire carcass; chemical analysis of some single representative cut; the yield of particularly fat or lean cuts; and the measurement of certain parts of the carcass, such as the thickness of fat on the shoulder, ham, or back.

The low density of fat when compared to other body components indicates that there should be a high relationship between the fat content and the specific gravity of a carcass. The purpose of this study was to investigate the possibility of using specific gravity to estimate the fat content of the pork carcass.

Material and Methods

Correlations were used to determine the relationship between different measures of fatness or leanness and the carcass specific gravity. The 66 carcasses used in this study came from two groups of hogs. Group I included 34 hogs from three inbred Duroc lines and their crosses, which were fed a standard ration on record of performance test. Group II included 32 individually fed Duroc hogs. The latter group was fattened on 4 rations differing only in fat content. The means and variations of the carcass characteristics are given in table 1.

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After reaching a weight of 215 to 235 pounds, the hogs were taken off feed for a period of 24 hours and dressed packer style. After chilling for at least 24 hours, the carcasses were weighed and the specific gravity determined. Both halves of the carcasses were divided into the wholesale cuts: ham, loin, belly, and shoulder. The shoulders were trimmed "New York style", the loins were trimmed as uniformly as possible, and the hams were skinned. Approximately ¼ inch of fat was left on the ham and the loin. All calculations were made from measurements and weights on both halves of the carcass, except those in table 3 which were made on only the right half of group II carcasses.

TABLE 1. MEANS AND STANDARD DEVIATIONS OF CARCASS CHARACTERISTICS

Item	Symbol	Mean	Standard Deviation	Coeff. of Variation
Shrunk weight (lbs.)	W	215.8	5.75	.027
Av. backfat thickness (in.)	BF	1.81	.20	.110
Lean area in ham face (sq. in.)	Ha	21.21	1.77	.084
Area loin eye (sq. in.)	La	5.39	.70	.130
Carcass length (in.)	L	28.92	.70	.024
Chilled weight (lbs.)	W	155.3	5.02	.032
Percent primal cuts	Pc	64.67	1.99	.031
Percent lean cuts	Lc	46.86	2.08	.044
Percent fat cuts	Fc	41.89	3.19	.076
Specific gravity	Sg	1.027	.007	.006
*Percent ether extract	EE	54.56	4.10	.075
*Percent moisture	M	31.82	3.06	.096
*Percent protein	P	10.10	1.01	.100
*Percent protein+ash	PA	12.94	1.23	.095
*Percent moisture (fat-free basis)		70.05	1.49	.028
*Percent protein (fat-free basis)		22.24	1.10	.049

* On right side of group II carcasses only.

Measurements used in this study were: average backfat thickness (BF), the average of 3 measurements on each side of the carcass taken at the level of the third rib, last rib, and sixth lumbar vertebra; area of lean in the ham face (Ha), calculated as a product of the width times the depth of lean; area of loin eye (La), calculated as the product of width times depth of eye muscle at the last rib; percentage primal cuts (Pc) (ham, loin, belly, and shoulder); percentage lean cuts (Lc) (ham, loin, and shoulder); percentage fat cuts (Fc) (belly and cutting fat); specific gravity (Sg); percentage ether extract (EE); percentage moisture (M); percentage protein (P); and percentage protein plus ash (PA). All percentage yields were calculated using chilled carcass weight.

Hydrostatic weighing as described by Rathbun and Pace (1945) was used to determine the specific gravity of the carcass. Each half of the carcass was weighed in air to the nearest .1 pound, then submerged in water and weighed to the nearest 5 grams. Weight in water was converted to pounds to the nearest third decimal. The weights of the two sides were added to give the weight of the entire carcass. The formula, $\frac{\text{weight in air}}{\text{weight in air minus weight in water}}$, was used to calculate the specific gravity of the carcass.

To weigh the carcass submerged in water, a tank six feet deep and two and one-half feet in diameter was placed on an elevator in the basement of the meat laboratory and raised until the top of the tank was about a foot above the level of the first floor. A 1500 gram Torsion balance was extended over the edge of a table placed across the first floor elevator opening. The tank was filled with water and the carcasses were suspended in the water from the balance by means of a heavy string with a metal hook attached to the hind foot. One side was weighed at a time.

Chemical determinations were made on samples of the entire right side of all carcasses in Group II. This was done by skinning, boning, and dividing the right side into fat, sausage (lean and fat), bones, and skin. Samples were taken of each of these items, chemical determinations were made, and the composition of the side calculated.

Results

The specific gravity values obtained for the pork carcasses in this study are lower and less variable than the values that have been obtained for guinea pigs and men (Rathbun and Pace, 1945; Behnke *et al.*, 1942). This difference may be explained by the greater fat content of the pork carcass as compared to guinea pigs and man, and by the uniformity in weight and condition of this particular group of hogs.

In general, the measures of leanness show smaller coefficients of variation than do the measures of fatness. These findings are in agreement with Dickerson (1947) and indicate that the fat content is more variable than the lean content of the carcass. The relatively greater variability in fat content is further shown when the composition of the right side of Group II carcasses is computed on a fat-free basis. On this basis the variability in percentage moisture was reduced by 71 percent and the variability in the percentage protein was reduced by 51 percent.

In table 2 intra-group correlations between specific gravity and area of loin eye, percentage primal cuts, percentage lean cuts, and carcass length, were positive and highly significant. Highly significant negative correlations were found between specific gravity and average backfat thickness and between percentage fat cuts and carcass weight.

Percentage of lean cuts was more highly correlated with specific gravity (.84) than it was with backfat thickness (—.72), area of the

TABLE 2. CORRELATIONS BETWEEN CERTAIN CARCASS MEASUREMENTS

Item [1]	BF	La	Pc	Lc	Fc	Ha	L	W
Sg	—.68**	+.46**	+.68**	+.84**	—.78**	+.22	+.56**	—.42**
BF		—.37**	—.67**	—.72**	+.69**	—.38**	—.62**	+.38**
La			+.41**	+.51**	—.47**	+.66**	+.37**	+.17
Pc				+.84**	—.60**	+.13	+.51**	—.29*
Lc					—.81**	+.52**	+.54**	—.35**
Fc						—.63**	—.61**	+.09

* =P. .05.
**=P. .01.
[1] See table 1 for description of symbols.

TABLE 3. CORRELATIONS OF CARCASS MEASUREMENTS WITH CHEMICAL ANALYSES

Item	BF	La	Pc	Lc	Fc	EE	P	PA	M	Ha
Sg	—.49**	+.68**	+.69**	+.78**	—.81**	—.75**	+.65**	+.72**	+.68**	+.43*
BF		—.54**	—.56**	—.70**	+.74**	+.48**	—.51**	—.58**	—.45**	—.50**
La			+.20	+.78**	—.80**	—.60**	+.60**	+.64**	+.54**	+.64**
Pc				+.71**	—.72**	—.67**	+.59**	+.64**	+.60**	+.65**
Lc					—.92**	—.67**	+.66**	+.77**	—.73**	+.85**
Fc						+.78**	—.65**	—.78**	—.69**	—.57**
EE							—.85**	—.87**	—.98**	—.44*
P								+.96**	+.76**	+.41*
PA									+.52**	+.40*

loin eye (.51), or lean area of the ham face (.52). Similarly, the percentage of fat cuts was more highly correlated with specific gravity (—.78) than with backfat thickness (.69), area of loin eye (—.47), or lean area of the ham face (—.63).

Correlations between the carcass measurements and chemical analyses of the right side of Group II carcasses are presented in table 3. Specific gravity was more highly correlated with the percentage of primal cuts, lean cuts, fat cuts, ether extract, and protein than was average backfat thickness.

It is of interest that the correlation between specific gravity and percentage of lean cuts is slightly higher than the correlation between the percentage of protein and the percentage of lean cuts (table 3).

Similarly, specific gravity was more highly correlated with percentage of fat cuts than with percentage of ether extract. Possibly errors in sampling the material for chemical analysis could account for the lower correlations between the yield of either fat or lean cuts and the chemical analysis than between the yield of these cuts and specific gravity. The principal difficulty in the chemical analysis was in obtaining a representative sample of the carcass. Errors in the separation of meat and bone, losses of moisture and fat in the cutting, grinding, mixing and sampling operations, and the difficulty of mixing materials of widely varying composition into a homogeneous mass all contributed to the sampling error.

TABLE 4. SPECIFIC GRAVITY AND CHEMICAL ANALYSES OF BONELESS MEAT

Kind of Sample	Sp. Gr.	Ether Ext.	Prot.	Mois.	Ash
Leaf fat	.948	93.42*	1.80	4.78	—
Belly 1	.946	64.44	7.50	27.75	.37
" 2	.976	80.13	4.41	15.30	.18
" 3	.979	81.32	4.94	13.63	.23
" 4	.995	63.36	8.59	27.76	.37
Boned ham 1	1.032	30.60	14.03	54.43	.61
" " 2	1.034	31.66	14.42	53.20	.75
" " 3	1.034	26.69	15.47	57.23	.84
" " 4	1.035	29.39	15.28	54.66	.70
" " 5	1.035	28.16	15.84	55.14	.73
" " 6	1.044	21.70	16.22	60.70	.83
Liver 1	1.069	2.35	19.68	72.96	1.74
" 2	1.075	1.22	20.62	71.93	1.45

* Chemical determinations were not made on this sample, but the average composition of three samples of leaf fat was used.

Partial correlations indicated that differences in carcass weights, as they existed in these data, had little effect on correlations between the various items measured.

Multiple correlations indicated that specific gravity combined with a number of measures of fatness or leanness were no more closely related to fat or lean content of the carcass than was specific gravity alone.

It appears that the actual leanness or fatness in the pork carcass was as accurately estimated from the percentage fat cuts or percentage lean cuts as from chemical determinations. Analysis of the data presented

indicates that there is little difference between the relationship of specific gravity, percentage of lean cuts or percentage of fat cuts to such other criteria of fatness or leanness as the percentages of ether extract, protein and moisture. If this is true, specific gravity could be used to estimate the fat content of the carcass as accurately as could the percentage fat cuts, and would have the advantage of providing the same information before the carcass is cut.

The specific gravities and chemical analyses of 13 samples of boneless meat are given in table 4. This table brings out the relationship between specific gravity and the percentage of ether extract and protein in samples of boneless meat varying widely in fat content. There was a correlation of —.95 between specific gravity and percentage ether extract and a correlation of +.95 between specific gravity and percentage protein.

Summary and Conclusions

Carcass data from two groups of hogs were studied to investigate the possibility of using specific gravity as a means of estimating the fat or lean content of the carcass. Group I included 34 hogs from 3 inbred Duroc lines and their crosses produced in the Oklahoma Swine Breeding Project. Group II included 32 individually fed outbred Duroc hogs used in a feeding trial at the Oklahoma Station. Hogs from both groups were slaughtered at weights ranging from 202 to 230 pounds.

The average specific gravity for the 66 carcasses was 1.027.

Intra-group correlations of specific gravity with area of loin eye, percentage primal cuts, percentage lean cuts, and carcass length, were positive and highly significant. Highly significant negative correlations were found between specific gravity and average backfat thickness, percentage fat cuts, and chilled carcass weight.

The correlations calculated in this study indicate that the fat or lean content of the carcass may be as accurately estimated by the specific gravity as by the percentage fat cuts or percentage lean cuts.

Partial correlations indicated that differences in carcass weights in these data had little effect on correlations between the various items measured.

Multiple correlations indicated that specific gravity combined with some of the other measures of fatness or leanness was no more closely correlated with the fat or lean content of the carcass than was specific gravity alone.

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A STUDY OF HETEROSIS IN SWINE[1]

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INCREASED vigor in offspring resulting from the cross of two inbred strains of corn or laboratory animals is an established fact. Superior performance in crosses of breeds of swine has been reported by Winters *et al.* (1935), Lush *et al.* (1939), Hutton and Russell (1939), Robison (1946, 1948), and others.

Inbred strains of swine have been in the process of development at several state experiment stations through cooperation with the Regional Swine Breeding Laboratory (Craft, 1943) since 1937. Winters *et al.* (1944) reported results of crossing inbred lines within the Poland China breed and between breeds. Line crosses between breeds gave a greater increase in vigor than line crosses within the Poland China breed. The better performing inbred lines produced superior crossbreds. Genetic diversity of the parental stocks appeared to be an important factor affecting the amount of increased vigor. When compared to the performance of outbred swine reared under favorable conditions, the line crosses showed an advantage in number of pigs weaned per sow, average weaning weight per pig, daily rate of gain and individual pig weight at 180 days.

Dickerson *et al.* (1946) compared the performance of single crosses between inbred lines of Poland Chinas with inbreds by the same boar. Mortality was lower among crosses than inbreds, both before and after birth, and at five months of age crosses exceeded inbreds by 1.4 live pigs per litter or by 42 percent. Crosses were 12 percent heavier at weaning time and exceeded inbreds by 25 pounds or 21 percent at 154 days. No advantage was shown for crosses in amount of feed required for 100 pounds of gain from 84 days of age to 225 pounds final weight.

The object of this study was to determine the effects of crossing inbred lines of swine developed by rigorous selection for characters of

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economic importance and to study factors contributing to the phenomenon of heterosis in swine.

Data and Methods

The basic data for this study were obtained from a breeding experiment with swine conducted by the Minnesota Agricultural Experiment Station in cooperation with the U. S. Regional Swine Breeding Laboratory. During the seven-year period covered by this study, 1941–1947 inclusive, performance data were gathered on 2213 pigs representing 373 spring-farrowed gilt litters. Included in this total were 42 litters and 322 pigs of the Minnesota No. 1 line; 52 litters and 348 pigs of the Minnesota No. 2 line; 141 litters and 797 pigs representing 9 different inbred Poland China lines; 67 litters and 350 pigs resulting from crosses of the inbred Poland China lines; and 71 litters and 396 pigs representing different cross combinations of the 3 breeds. These records were gathered from five different stations distributed throughout the state. Environmental conditions varied from year to year and station to station. In several instances, the parental lines were reared at a different location than the crosses. There was no reason to believe, however, that the environment was more favorable or more unfavorable for the crosses or the parental lines. Consequently, the data studied represents the average performance of the parental lines and crosses of these lines. The methods of keeping records, the data collected, feeding and managerial practices and the breeding program are all described by Winters *et. al.* (1943).

For this study, three measures of vigor were used; namely, (1) weaning weight, (2) rate of gain during the post-weaning period, and (3) efficiency of feed utilization during the post-weaning period.

The standard age for weaning throughout the project was 56 days. Weight at weaning is a measure of both the milking capacity of the sow and an index of hereditary growth impulse. In this study, it is assumed to be more a measure of the latter since the breeding of the dams of the crossbred litters was essentially the same as that of the dams of the inbred litters.

Rate of gain from weaning to 154 days of age is a valid measure of inherent ability to grow, assuming a suitable environment. The use of two measures of growth is justified on the grounds that two distinct types of environment are involved.

A number of factors must be taken into consideration in making critical comparisons of efficiency of feed utilization. In the first place,

maintenance requirements increase with increased weights. Since all the pigs in this study were weighed out at a constant age, with varying weights, feed requirement was corrected to a standard final weight of 200 pounds. Similarly, initial weight may affect the feed required per hundred pounds gain. Therefore, an adjustment was made so as not to handicap the heavier weaning pigs. The correction used in this study was based on a table prepared by Evvard *et al.* (1928).

Since the relative values of these three factors, weaning weight, rate of gain, and efficiency of feed utilization, have not been determined, they are taken as equal in this appraisal of vigor. The advantage or disadvantage of the crossbreds in comparison with the average of the

TABLE 1. SAMPLE OF METHOD OF DETERMINING INCREASE IN VIGOR OF CROSSES

Item	Fx of Pigs, %	Measure of Vigor			Ave. of Three Measures
		Weaning Wt. lbs.	Daily Gain, lbs.	Feed per 100 lbs. gain lbs.[1]	
BS—V Cross	9.0	33.8	1.56	306.0	
Parental Stock Av.	35.6	31.7	1.38	325.0	
Cross Advantage	—26.6	2.1	0.18	19.0	
Percent Advantage		6.7	13.0	5.8	8.5

[1] Corrected to 200 lbs. final wt. and zero difference in weaning wt.

parental stock was calculated for each of the measures in terms of percent. The results were added for each cross and divided by three to give the overall increase or decrease in vigor of each group of crossbreds. This is similar to the method of Winters *et al.* (1944). A sample of the data is shown in table 1.

Results and Discussion

The results obtained in this study are in essential agreement with those of other investigations in demonstrating the relationship between genetic diversity of parental stocks and heterosis. These results are shown in table 2.

For this comparison, similar types of matings were grouped together. In other words, the inbred Poland China line crosses include the two-, three-, and four-line crosses. The #2—inbred Poland China line crosses include both the first crosses and backcrosses. First crosses, backcrosses

and crisscrosses of the #1 and inbred Poland China lines were grouped together as were the #2—#1 first crosses and backcrosses.

The origin of the parental stocks is described by Winters *et al.* (1943). The #2 line was developed from a cross of Yorkshire and two inbred Poland China lines. There is no way of determining the contribution made by either of the parental breeds to the genetic composition of the #2 line. However, it is entirely reasonable to assume that the inbred Poland China lines and the #2 line do have more genes in common than the #1 line and the Poland China lines, or the #1 and #2. This is indicated by the amount of heterosis in #2—Poland China line crosses as compared to #1—Poland China and #2—#1 crosses.

TABLE 2. DECREASE IN Fx AND INCREASE IN VIGOR RESULTING FROM CROSSES OF PARENTAL STOCKS OF DIVERSE GENETIC ORIGIN

Cross	Av. Decrease in Fx percent	Av. Increase in Vigor percent	Percent Increase in Vigor for each 10% decrease in Fx
Inbred Poland China lines	29.7	10.5	3.6
#2—Inbred Poland China	28.7	12.4	4.4
#1—Inbred Poland China	31.5	18.2	5.9
#2—#1	20.4	21.0	11.3

TABLE 3. COMPARISON OF PERFORMANCE OF M AND V LINES AND CROSSES INVOLVING THEM

Observation	M	V	M-V	1-V	1-M	2-V	2-M
Inbreeding—%							
of litters	74.6	38.4	0.0	0.0	0.0	0.0	0.0
of dams	72.2	33.9	55.3	31.4	60.5	38.0	75.0
Pig weight—lbs.							
at 56 days	32.5	28.1	34.3	41.7	44.5	38.7	35.9
at 154 days	141.4	153.0	161.0	204.2	198.5	192.6	189.5
Daily gain—lbs.	1.11	1.27	1.27	1.66	1.58	1.55	1.53
Feed per 100 lbs. gain—lbs.[1]	329.0	325.0	323.0	324.0	313.0	284.0	331.0

[1] Corrected to 200 lb. final weight.

The performance of crosses of the M and V inbred Poland China lines contribute evidence indicating similarity of genic complexes in

these two lines. Table 3 shows the performance of these two lines and crosses involving them.

When the M and V lines were crossed, there was only a slight improvement in performance as compared with the parental stock. This would indicate that the same basic genes responsible for performance in the M and V lines are essentially identical. This assumption is supported by results from crosses of the #1 and #2 lines with the M and V lines. The results are essentially identical. Chance fixation of these genes must have occurred since the M and V lines have been maintained as separate lines since they were started—the M line in 1924 and the V line in 1938—from stock which showed no special relationship other than the fact that they were registered Poland China hogs. On the basis of their origins, it would be expected that these two lines should differ about as much as could be expected of two lines within the Poland China breed. These results also give some indication of the degree of genetic purity required to obtain maximum hybrid vigor. As measured by the coefficient of inbreeding, the M line possesses approximately two times as much genetic purity as the V line. However, the two lines perform essentially the same in crosses, with the V having a slight advantage.

From the standpoint of practical application the question is not how much advantage the line-crosses show over the inbred lines but how they compare in performance to hogs produced by conventional methods. The most desirable method of making this comparison would be to test the two groups under similar environmental conditions. The facilities required to conduct such a test which would include an adequate sample of all the breeds would be out of all proportion to those now available. Consequently, for this comparison, the average performance of the better combining inbred line crosses was compared with the average performance of non-inbreds and non-inbred breed crosses reared under favorable environmental conditions as reported by investigators from a number of midwestern experiment stations and by the U.S.D.A. The comparison of the two groups is shown in table 4.

Use of the average performance of the better combining inbred line-crosses for this comparison is justifiable since it portrays more accurately the results which can be obtained and because a part of any breeding program, designed to improve performance, is concerned with isolating the lines which do give superior performance. It should not be inferred from this that the best combination has been found. With practically unlimited possibilities for recombination, it is not likely that such a combination will be found in the near future.

The results of this study indicate that the manifestations of heterosis in swine are what would be expected by increasing the efficiency of metabolism. They are the kind of results derived from a superior developmental system and once this superiority has been established, its apparent degree may be increased as manifested by the fact that vigorous animals may be much less susceptible to the effects of varying environmental conditions.

The genetic mechanism responsible for heterosis in swine is by no means any more clearly defined than in plants. As stated by Hayes

TABLE 4. COMPARISON OF PERFORMANCE OF BEST INBRED LINE CROSSES WITH NON-INBREDS AND BREED CROSSES

	Measure of Performance			
	Weaning Weight lbs.	Rate of Gain lbs.	Feed per 100 lbs. Gain lbs.[1]	Av. of Three Measures
Line Crosses	41.1	1.65	325.0	
Non-inbreds and Non-inbred Breed Crosses[2]	34.5	1.38	344.0	
Line Cross adv.	6.6	.27	19.0	
Percent adv.	19.2	19.6	5.8	14.9

[1] Corrected to 200 lb. final weight.
[2] U.S.D.A. Circ. 570, 698, 532. U.S.D.A. Tech. Bul. 836. Iowa Agr. Expt. Sta. Bul. 380. Ohio Agr. Expt. Sta. Bul. 242 (Bi-Monthly). Ohio Agr. Expt. Sta. Bul. 675. Okla. Agr. Expt. Sta. Tech. Bul. 7. Mo. Agr. Expt. Sta. Res. Bul. 413. Unpublished data, Minn. Agr. Expt. Sta.

and Immer (1942), "The lack of agreement among the various studies indicates that heterosis is manifested in various ways in different hybrids and that it may be due to various causes."

It is entirely reasonable to assume an explanation of some hybrid vigor on the basis of suppression of unfavorable recessives. Acceptance of this hypothesis would mean that "the hybrid represents the 'normal' or biological optimum and that the parents are inferior types—they contain an accumulation of deleterious factors". Whaley (1944).

The relationship of genetic diversity to heterosis tends to favor East's (1936) "multiple allelic series" hypothesis. The crossing of genetically diverse parents increase the chances of bringing together genes which complement each other and which may never be paired in either parental line for the simple reason that both do not occur in either parental population.

Fortunately, the development of methods of breeding directed toward obtaining the full benefits of hybrid vigor are not necessarily dependent upon a single, definitive explanation of the genetic mechanism involved. Considering heterosis as a phase of quantitative inheritance, which is itself Mendelian in nature, serves as a basis for the development of such methods.

Summary

A study was made of the results obtained in crossing inbred lines of swine which were developed by following a flexible system of inbreeding and rigid selection for characters of economic importance. Average inbreeding of the lines, which included nine inbred lines of Poland China and two lines based on crossbred foundations (Minnesota No. 1 and Minnesota No. 2) ranged from 22 percent to approximately 75 percent.

An overall estimate of hybrid vigor was determined by averaging the advantage, in percent, of three measures of vigor, (1) weaning weight, (2) rate of gain and, (3) efficiency of food utilization, of the crossbred pigs over the average of the parental lines.

Crosses of inbred lines within the Poland China breed showed less heterosis than crosses of Minnesota No. 1 and Minnesota No. 2 or crosses of the Poland China lines and either Minnesota No. 1 or Minnesota No. 2. These results are interpreted as an indication of the importance of genetic diversity in relation to heterosis.

Similarity of genic complexes in two inbred lines of Poland Chinas is indicated by the performance of crosses involving them. On the basis of their origins, it would be expected that these two lines should differ about as much as could be expected of two lines within the Poland China breed. The performance of these two lines in crosses also gives some indication of the relationship between genetic purity and hybrid vigor. As measured by the coefficient of inbreeding, one line possesses approximately two times as much genetic purity as the other line. However, the two lines perform essentially the same in crosses.

A comparison of the performance of the better combining inbred line crosses with that of hogs bred by conventional methods (data composited from the literature) showed an advantage of approximately 15 percent in favor of the inbred line crosses. This indicates that the methods employed in developing the inbred lines have been effective in bringing about a certain degree of purification for the more desirable genes.

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THE PHYSIOLOGICAL EFFECTS OF A FAT-DEFICIENT DIET ON THE PIG[1]

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IT has been generally agreed that the rigid exclusion of fat from the diet of the rat will cause a fat deficiency disease. During the past 20 years much work has been done to determine the curative agents and the amounts of these agents required to cure this disease.

Modern methods used in the extraction of fat and oil from commercial feeds commonly used for swine production have resulted in rations of lowered fat content. Therefore, the knowledge of the effects of low-fat and fat-deficient rations on swine will be valuable for the selection of feeds to be used in preparing efficient swine rations.

It was 1929 before an adequate synthetic diet was prepared with little enough fat to demonstrate the nutritional significance of this nutrient. Burr and Burr (1929) described a new nutritional disease in the rat produced by the rigid exclusion of fat from the diet. Later White *et al.* (1943) produced essentially the same symptoms in albino mice by excluding the fat from the diet. In 1947 Beeson and Kennelly (1947) produced fat deficiency symptoms in growing pigs. This deficiency disease was characterized by dissatisfaction with the diet, scaliness of the skin, poor hair growth, increased amounts of hyalin-like materials in the kidneys, and low red and white blood cell counts.

This study was initiated to determine if fat is required for growth and development of swine and to study the physiological effects of low fat diets on weanling pigs.

Experimental

This investigation included two experiments. Experiment I was conducted in the spring of 1948, and Experiment II was conducted in the winter of 1948–49. In each experiment eight weanling Duroc pigs were obtained from the Purdue Livestock Experimental Farm. Litter mates were paired according to sex and initial weight. One male and

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[3] The authors express their appreciation to Dr. L. P. Doyle for conducting the autopsies on the experimental pigs.

one female from each litter were individually-fed the fat ration, and their paired mates were fed the fat-free ration. The rations given in table 1 were fed twice daily in amounts to satisfy the individual appetite of each animal. The vitamin supplement was made into a solution by adding 30% ethyl alcohol and the concentration was regulated so that

TABLE 1. COMPOSITION OF DIETS

Ingredients	Percent	
	Fat Diet	Fat-Free[3] Diet
Casein	27.0	27.0
Lard	5.0	0.0
Dextrose	60.8	65.8
Salt Mixture[1]	5.2	5.2
Vitamin Supplement[2]		
Vitamin B_{12} Concentrate[4]	0.5	0.5
Liver Extract[5]	0.5	0.5

[1] Salt Mixture:

	Percent
Potassium chloride	6.7
Dicalcium phosphate	46.3
Sodium chloride	13.5
Calcium carbonate	16.9
Magnesium carbonate	8.5
Potassium phosphate	5.7
Iron pyrophosphate	2.100
Manganese chloride	0.020
Copper sulfate	0.020
Zinc oxide	0.016
Cobalt carbonate	0.016
Potassium iodide	0.228
	100.000

[2] Vitamin Supplement:

(amounts daily per kg. body weight in gm.)	
Thiamine	0.50
Riboflavin	0.12
Niacin	1.20
Pyridoxine	0.20
Choline	10.00
Calcium pantothenate	0.50
(per pig daily in mg.)	
Alpha tocopherol	50.0
Menadione	2.0
Delsterol	200.0
Vitamin A	5000 I.U.

[3] In Experiment II the casein was "Vitamin Test Casein" which was produced by Nutritional Biochemicals Corp., Cleveland, Ohio. Also at the beginning of the fifth week of Experiment II, six percent casein was replaced with dextrose.

[4] Vitamin B_{12} Concentrate, produced by Merck and Co., Rahway, New Jersey, was used to replace equal amounts of dextrose in the diets of Experiment II during the sixth week.

[5] "Lilly's Liver Extract" produced by Eli Lilly and Co., Indianapolis, Indiana, replaced equal amounts of dextrose in the diets of Experiment II starting the seventh week and continued to the end of the experiment.

each pig received 10 cc. of the mixture in the evening feed each day. The ration was mixed in 50-pound amounts which lasted for about three days, and the vitamin supplement was mixed every two weeks and stored at about 0° C. The pigs were watered in individual troughs, *ad libitum*.

The pigs were kept in individual adjacent pens on raised wire screen floors. In the summer the pens were washed thoroughly twice daily and the pigs were sprinkled with water at feeding time to make them more comfortable. In the winter the pens were cleaned daily and heat lamps

above each pen were used to make the pigs more comfortable and to keep the water from freezing.

The diets used were modifications of the one used by Heinemann *et al.* (1946). They were modified by the substitution of dextrose for sucrose, the addition of celluflour instead of treated oat hulls to supply bulk, and in Experiment II the addition of dried liver extract (Lilly's) supplied unidentified growth factors. The crude casein used in Experiment I had a low fat content and the entire diet contained about 0.12% of ether-extractable material. The casein used in Experiment II was extracted with hot alcohol and contained 0.06% ether-extractable material. Both of these low fat diets will be referred to in this paper as fat-free diets. Vitamin A was fed as a fish oil concentrate with a potency of 500,000 I.U./gram.

Each week the pigs were weighed individually. The feed was weighed at the time of feeding. The small amount of feed wasted could not be determined, but appeared to be about the same for each pig.

Blood samples were taken at two-week intervals and analyzed for plasma vitamin A, hemoglobin, red blood cells, white blood cells, plasma cholesterol, and plasma lipids. Post mortem examinations were made on each pig in Experiment II. Histological comparisons were made, abnormalities noted, and vitamin A and fat content of the livers were determined, and the iodine number of the back fat was determined.

Methods used for laboratory determinations were as follows: the blood plasma vitamin A was determined by the method described by Kimble (1939). The procedure of Evelyn and Mallory (1938) was used to determine the amount of hemoglobin present in the blood and to obtain the blood plasma cholesterol values, the method of Pijoan and Walter (1937) was used. Red and white blood cell counts were made as suggested by Gradwohl (1938); the level of blood plasma fat was determined by the method of Allen (1934) and to determine the vitamin A content of the liver the procedure of Gallup and Hoefer (1946) was used. The Hanus iodine number, the moisture and the fat of the liver were determined as prescribed by the A.O.A.C. (1945). Statistical significance was determined by using methods described by Snedecor (1946).

Results and Discussion

External Symptoms

Fat-deficiency symptoms were noted in both Experiment I and Experiment II; however, the symptoms in Experiment II were more severe. This is believed to be a result of the lower fat content of the

diet used in Experiment II. It was noted that fat-deficiency symptoms began to appear after 42 days on the fat-free diet and after 63 days the symptoms became quite severe. Although scaly dandruff appeared over all of the body, it was more concentrated on the tail, back and shoulders. Another symptom was the loss of hair starting on the lower part of the hind legs and gradually spreading along the belly, sides and back (compare figures 1 and 2). The hair remaining on the body was dull and dry and it lacked the luster of the hair on the pigs receiving fat. Both dandruff and loss of hair appeared to be greater in the male pigs than it did in the female pigs. A brown gummy exudate appeared on the belly and sides of the pigs. The pigs receiving fat in their diets had a more thrifty appearance than the pigs receiving the fat-free diet. Typical fat-deficiency necrotic areas were noted on the shoulders and necks of the pigs receiving the fat-free diet. There was no difference in the degree of fatness of the pigs receiving the fat-free and 5% fat diet. The fat deficiency symptoms of the pig are almost identical to those reported by Burr and Burr (1929) in rats on a fat-deficient diet.

After 63 days on the fat-free ration, one male and one female pig were given 1.5% corn oil which replaced an equal amount of dextrose in their diet. Following this treatment, the dandruff condition was alleviated, the skin became more oily, and the pigs appeared to be more thrifty. No new hair growth appeared, but the loss of hair did stop and the necrotic areas were replaced with new tissue.

Growth Rate

Feeding results on different levels of fat are summarized in table 2. It may be noticed that in Experiment I the fat content in the diet had little effect on the growth rate, while in Experiment II the amount of fat in the rations had a very marked effect on the growth rate. This difference in growth rate is believed to be a result of the difference in the fat content of the two diets to supply essential fat or fatty acids for normal growth in the pig. Although the fat content of the diet used in Experiment I was only 0.12%, apparently essential fatty acids were present in sufficient amounts to meet the minimum requirement of fat for growth in the pig. On the other hand, the diet used in Experiment II containing 0.06% did not meet the minimum requirement for fat to support normal growth in the pig. It should be pointed out that the excellent growth obtained in Experiment I is ample evidence that the purified diets used in this study are nutritionally adequate to support growth in the weanling pigs.

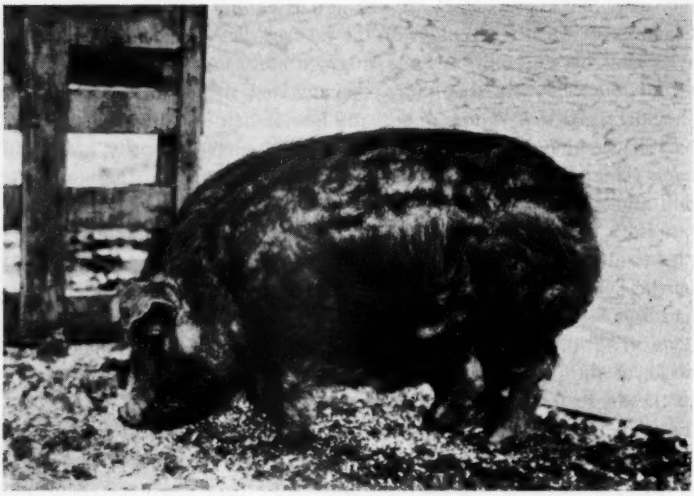

Figure 1. A male pig receiving 5.0% fat diet (litter mate to the pig shown in Figure 2).

Figure 2. Effect of a fat-free (0.06% ether-extract) diet. Note loss of hair, scaly dandruff-like dermatitis, especially on the feet and tail.

The results obtained in Experiment I are contrary to those obtained by Shrewsbury and Vestal (1946). They found that rations containing only 0.5% fat produced a slower rate of gain than those containing 5 to 20% fat. Robinson (1943) using a natural diet supplemented with maize oil and coconut oil to alter the fat content, found that the larger the fat content the greater were live weight gains regardless of whether full feeding or limited feeding was practiced. It is possible that the difference in results of this study and the latter two studies was due to the different diets used. In our study a purified diet was used

TABLE 2. COMPARISON OF GROWTH RATE AND FEED CONSUMPTION

Items Compared	Experiment I		Experiment II	
	Fat Diet	Fat-Free Diet	Fat Diet	Fat-Free Diet
Number of pigs	4	4	4	4[1]
Days on experiment	56	56	77	77
Average initial weight, lb.	36.1	35.0	48.2	48.2
Average final weight, lb.	125.5	121.9	130.2	106.0
Average gain, lb.	89.4	86.9	82.0	57.8
Average daily gain, lb.	1.60	1.55	1.06	0.81
Average daily feed, lb.	4.10	4.06	3.80	3.87
Feed consumed per lb. of gain, lb.	2.57	2.62	3.57	4.83

[1] One male and one female pig were taken from the fat-free diet at the end of the ninth week. There were only two pigs on this diet for the remaining two weeks.

with the fat being supplied in the high fat diet by lard while in the studies of Shrewsbury and Vestal (1946) and Robinson (1943) the fat was supplied by maize oil, coconut oil, and soybean oil and a more or less natural diet was used. Even though the results of this study are contrary to those of other workers, these data indicate that a ration of this type containing only 0.12% of fat will support as good growth as a ration containing 5% fat supplied by lard. Apparently the fat requirement of the pig for growth is very low.

In Experiment II, the pigs receiving fat in their diet had a significantly ($P<.01$) greater growth rate than their litter mates receiving no fat. After 35 days on experiment both groups showed a considerably decreased growth rate. This decrease was believed to be caused by the

loss of unknown factors in the hot alcohol extraction of the casein; therefore, 0.5% vitamin B_{12} concentrate (Merck's) was incorporated in the ration by replacing an equal amount of dextrose. This addition to the diet caused an increase in the gain of the pigs receiving fat, but did not appear to increase the gain of the pigs on the fat-free diet. On the 42nd day of the experiment 0.5% "Lilly's Dried Liver Extract" was used to replace the vitamin B_{12} concentrate. The addition of the liver extract to the diet promoted a further increase in the daily gain of the pigs receiving fat and stimulated a like increase in the gain of the pigs on the fat-free diet; therefore, the liver extract was used the remainder of the experiment.

After 63 days the fat-deficient pigs in Experiment II had almost stopped growing. They were gaining from 0.1 to 0.3 pound daily. This cessation of growth was believed to be a result of inadequate fat or fatty acids in the diet, so one male and one female were given 1.5% corn oil replacing an equal amount of dextrose in the fat-free ration for the remaining three weeks. These two pigs immediately resumed a normal growth rate, 1.0 to 1.1 pounds daily, and continued gaining at this rate until the end of the experiment. The pigs on the fat-free diet receiving no supplementary corn oil continued for 7 more days without gaining weight. The following 7 days they lost about 1 pound per day and after 77 days on the fat-deficient diet these pigs died.

The 42 days required for the deficiency symptoms to appear in the pig compare favorably to the time required to deplete the rat and mouse. White *et al.* (1943) reported that the fat deficiency symptoms appeared in 7 weeks in the rat and in 5 weeks in the albino mouse. The pigs on the low-fat diet reached a growth plateau when their gains were about 80% of those of the controls. Burr and Burr (1929) reported cessation of growth in rats on a low-fat diet when the rats were about 25% under weight in comparison with the controls receiving fat. This plateau in the weight curve was maintained over a period of a few weeks to several months when the rats began to decline and invariably died unless given a curative dose of fat. Unlike the rats, the pigs maintained the plateau in the growth curve for only 2 weeks, then began to decline. After 1 week of losing weight both pigs died. Burr and Burr (1929) reported a more rapid loss of weight in the male rats than in the female rats, this sex difference was not noted in the fat deficient pigs.

When the pigs in Experiment II reached the growth plateau after 63 days on a fat-free ration, 1.5% corn oil was added to the diet of one male and one female to see if the skin condition and growth would

improve. Corn oil was fed at the rate of 1.5% of the diet because it contained approximately the amount of linoleic acid as recommended by Turpeinen (1938) to cure rats suffering from fat deficiency. The rats cited by Burr and Burr (1929) completely recovered from fat-deficiency symptoms in 10 weeks of treatment. The pigs in this study only partially recovered from the fat-deficiency symptoms when corn oil was added to their diet. Since these pigs were treated for only 3 weeks, it is possible that there may have been complete recovery in a longer period of time.

Ellis and Zeller (1930) reported that the lack of fat did not appear to exert material influences on the degree of fatness. The observation reported earlier that there was no difference in the degree of fatness noted between the pigs receiving fat and those receiving no fat confirms the work of Ellis and Zeller (1930).

There was no significant difference in the feed consumption of the various groups of pigs in this study. This is in agreement with the feed consumption of rats as reported by Burr and Burr (1930). It should be pointed out that the death of the two pigs on the fat-free diet was not due to the lack of feed, as they consumed about 3 pounds the day before they died.

Blood Analyses

Since the blood analyses in Experiment I were similar to those in Experiment II, only those in Experiment II will be reported and discussed. The blood analyses for Experiment II are summarized in table 3.

A very striking difference was noted in the vitamin A content of the blood plasma. The vitamin A level was significantly higher in the animals on the fat-free diet. Since both groups of pigs received the same daily intake of vitamin A, the differences were a result of the nature or effects of the diets. Sumner and Sumner (1940) showed that the unsaturated fatty acids had an antagonistic effect on carotene and vitamin A. In some instances, it has not been clear whether the destructive effects have been due to inhibition or actual destruction of vitamin A by oxidation caused by products of fat rancidity (Sherman, 1941). It seems very unlikely that the fat in this diet could become rancid before consumption. Also the vitamin A was consumed in a short time after it was mixed with the ration each day. It is possible that fat in the ration of the controls inhibited the absorption of vitamin A in some manner. If this is true, it is contrary to reports of Russell and coworkers (1942) who reported that the absorption of carotene by hens was about

TABLE 3. BLOOD ANALYSES

(Experiment II)

	Fat Diet (5%)	Fat-Free[1] Diet (0.06%)	Fat-Free Diet[2] Supplemented With 1.5% Corn Oil
Average Vitamin A (micrograms per 100 ml.)			
Initial	8.5	9.9	
16 days	15.6	21.4	
30 days	11.2	14.6	
44 days	16.3	20.7	
58 days	16.1	20.3	
72 days	11.1	7.9	8.9
86 days	9.7		13.8
Average Hemoglobin (gm. per 100 ml.)			
Initial	12.8	13.1	
16 days	11.6	12.3	
30 days	12.1	11.7	
44 days	11.6	11.8	
58 days	12.5	14.3	
72 days	14.3	14.2	14.9
86 days	14.5		13.8
Average Cholesterol (mg. per 100 ml.)			
Initial	102.0	106.8	
16 days	114.2	119.2	
30 days	94.0	77.0	
44 days	109.8	128.0	
58 days	96.2	102.8	
72 days	130.0	125.5	100.0
86 days	103.5		135.0
Average Red Blood Cells (thousand per cu. mm.)			
Initial	7,768	7,657	
16 days	7,380	7,817	
30 days	6,562	6,742	
44 days	6,375	7,137	
58 days	6,827	6,707	
72 days	7,355	7,155	7,945
86 days	8,235		7,220

[1] One male and one female pig were taken from the fat-free diet at the end of the ninth week. There were only two pigs on this diet for the remaining two weeks.

[2] One male and one female pig on the fat-free diet were given 1.5% corn oil in their diet at the end of the ninth week and this treatment was continued to the end of the experiment.

TABLE 3.—*Continued*

	Fat Diet (5%)	Fat-Free[1] Diet (0.06%)	Fat-Free Diet[2] Supplemented With 1.5% Corn Oil
Average White Blood Cells (per cu. mm.)			
Initial	24,362	24,200	
16 days	16,325	17,875	
30 days	15,350	15,350	
44 days	14,900	18,288	
58 days	18,638	13,825	
72 days	17,538	12,575	16,800
86 days	20,725		18,675
Average Blood Plasma Fat (mg. per 100 ml.)			
Initial	194	196	
16 days	149	130	
30 days	145	118	
44 days	158	125	
58 days	175	136	
72 days	168	135	155
86 days	148		150

60% of the intake of the ration containing approximately 4% fat, in contrast to 20% absorption in a ration extracted to 0.07% of this nutrient. They also found that fat promotes the absorption of vitamin A. Even though the vitamin A blood level of the control pigs was not as high as that of the fat-deficient pigs, it is apparently normal since they did not show any symptoms of avitaminosis A.

Fat intake apparently has no effect on the hemoglobin content of the blood, since the fat-deficient pigs had a lower content in Experiment I and generally higher content in Experiment II than the controls. The hemoglobin levels found in this study were quite similar to the mean value of 11.95 grams per 100 ml. of blood found for the pig as cited by Dukes (1942). Even though the hemoglobin present was adequate for normal growth, the addition of a vitamin B_{12} concentrate to the diet caused a significant increase in the hemoglobin value. There was a further increase with the addition of liver extract and the hemoglobin value continued to increase to the end of the experiment. The addition of corn oil to the fat-free ration had no significant effect on the hemoglobin level of the blood.

Bender and Maynard (1932) reported that a strong parallelism exists between the total lipids, the phospholipids, and the cholesterol of the blood. There was some resemblance in the curves of the blood cholesterol and total lipids in Experiment I; however, there was none in Experiment II. The variation of the blood cholesterol appeared to be affected by factors other than the treatments of this study.

There was considerable variation of the red blood cells during this experiment. Some of this variation was probably due to the counting technique. The red blood cells were more abundant in the pigs receiving fat, especially near the end of the experiment. The red blood cell count varied from 6,375,000 to 7,945,000 cells per cubic mm. of blood. This range compares favorably with the mean for the pig of 7,440,000 red blood cells per cubic mm. of blood as reported by Dukes (1942). In Experiment II there was a sharp rise of red blood cells when vitamin B_{12} concentrate was added to the ration. As with the hemoglobin content this rise was increased with the addition of liver extract and the number of red blood cells continued to increase to the end of the experiment. The addition of 1.5% corn oil to the pig on the fat-free diet caused a rise in the number of red blood cells.

Fat may have an effect on the white blood cells. The white cells became progressively less in the pigs on the fat-free diet. Dukes (1942) reports that the mean white blood cell count of pigs in 17,110 cells per cubic mm. of blood. This compares favorably with the white blood cell range of 12,575 to 24,362 white blood cells per cubic mm. of blood found in this study. The addition of corn oil to the diet of the fat-deficient pigs caused a considerable increase in the number of white blood cells.

It is evident that the level of fat in the diets of these pigs has an appreciable effect upon the lipid content of the blood plasma. Boyd (1944) reported that the total lipid of the red blood cells is fairly constant at about 5% in all species in spite of the wide variation in the concentration of plasma lipids. He suggests that plasma or serum should be selected for lipid analysis rather than the whole blood if only one analysis or set of analyses is to be done. Blood plasma was used for the total lipid determination. Maynard and McCay (1929) reported that in the dairy cow the blood lipid concentration was markedly influenced by changes in the amount of the fat-intake, the changes being in general in the same direction. These results in dairy cattle are quite similar to those found in the pigs in this study. The pigs fed the fat-free diet had a significantly lower blood-fat level than the pigs receiving the 5% fat diet. The addition of corn oil to the fat-free diet raised the blood-fat level of the pigs.

Post Mortem Examination

After 77 days both pigs on the fat-free diet died. At this time the two control litter mates were killed. The following week, after 84 days on experiment, the fat-deficient pigs treated with 1.5% corn oil and their control litter mates were killed. All pigs were examined for any physiological abnormalities of the various organs and tissues.

Post mortem macroscopic examination of the fat-deficient pigs revealed the following characteristic abnormalities: underdeveloped digestive system with a small amount of coagulated fibrous material in the abdomen and congestion of the stomach and colon, under-developed gall bladder with little or no bile present, slightly enlarged thyroid glands and some hemorrhage and necrosis of the kidneys.

The only abnormality noted in the fat-free pigs that received a curative dose of 1.5% corn oil in their diets was the small amount of hemorrhage and necrosis in the kidney. The controls showed no abnormalities.

The most striking abnormality noted in the post mortem macroscopic examination was the abnormally small gall bladders of the pigs on the fat-free diets. There was only a small amount of bile present in the gall bladder. Dukes (1942) points out that bile is secreted continuously by hepatic cells; however, the rate varies with the food and other factors. It is not known what causes the secretion of bile, but Dukes (1942) states that a number of observations made it likely that a humoral mechanism is concerned and it is believed that bile salts and secretin absorbed from the intestine are the excitants. The production of bile under the influence of stimuli coming from the seat of most active digestion (small intestines) would thus be coordinated with the need of the digestive organs. Schmidt and Ivy (1937) reported that the gall bladder of the pig had little or no concentrating ability; therefore, the main function of the gall bladder in the pig is to store bile and to regulate its secretion into the gut. Since there was little or no bile present in the gall bladder, it would indicate that there was little secretion of bile by the liver. It may be possible that the fat content of the diet was so low that bile was not needed for digestion and its secretion had practically stopped. The pigs on the fat-free diet given 1.5% corn oil for three weeks, had gall bladders comparable in size to those of the controls. This would indicate that the function of the gall bladder was not permanently injured by the fat-free diet, and that when the fat was increased in the diet the gall bladder would resume its function.

The digestive systems of the fat-deficient pigs appeared underdeveloped when compared with those of the controls; however, there is no explanation why this should be so, since the feed had the same content of fiber and the feed consumption was about the same in both groups.

The presence of enlarged thyroids of the fat-deficient pigs confirm the results by Beeson and Kennelly (1947).

The hemorrhage and necrosis found in the kidneys of the pigs on the fat-free ration was not severe and apparently did not interfere with

TABLE 4. AVERAGE WEIGHTS OF THE TESTES, OVARIES, AND THYROIDS

(Experiment II)

Items Compared	Treatment		
	Fat Diet (5%)	Fat-Free[1] Diet (0.06%)	Fat-Free[2] With 1.5% Corn Oil
Weight of testes, gm.	118.4	55.5	122.2
Weight of testes as % of total live weight	0.23	0.13	0.20
Weight of ovaries, gm.	2.0	1.0	6.2
Weight of ovaries as % of total live weight	0.003	0.002	0.009
Weight of thyroid, gm.	18.3	26.2	18.1
Weight of thyroid as % of total live weight	0.03	0.06	0.03

[1] One male and one female pig were taken from the fat-free diet at the end of the ninth week. There were only two pigs on the diet for the remaining two weeks.

[2] One male and one female pig on the fat-free diet were given 1.5% corn oil in their diet at the end of the ninth week and this treatment was continued to the end of the experiment.

their normal function. Burr and Burr (1930) reported that rats on a low-fat diet had kidney damage, but apparently the damage was more severe since the degeneration was so great that blood was passed in the urine. The kidney damage in the fat-deficient pigs was apparently permanent since the addition of corn oil to their diets did not alleviate the symptoms.

The pigs in this study were approximately 5 months of age at the time of death, and they should have been sexually mature; however, it may be noted from table 4 that the ovaries and the testes were smaller in the pigs receiving the fat-free diet than the control pigs

receiving fat, and the fat-deficient pigs receiving 1.5% corn oil. Microscopic examination of the testicles revealed spermatogenic activity, but sperm were found only in the control males. The ovaries appeared to be normal in all the females, but only the control females had lutein tissue present in the ovary. The female pigs on the fat-free diet did not show any lutein cells indicating that they had not ovulated. The work of Burr and Burr (1930) indicates that the male sexual system of rats is affected by the lack of fat in the diet. The male rats of the fat-free diet did not mate and lost their sex drive. This condition was corrected by the addition of fat to their diets. Burr and Burr (1930) reported that female rats on a fat-free diet had irregular ovulation and in some cases ovulation ceased entirely. When a curative oil was fed, ovulation was resumed in a few days in rats. The testicles and ovaries of the pigs on the fat-free diet and fat-deficient pigs fed 1.5% corn oil were normal, but maturity was partially retarded by lack of fat in the diet. The reproductive organs of the pigs fed a curative dose of 1.5% corn oil in their diet were more mature than their litter mates receiving no corn oil.

Histological examination of sections of the skin from the pigs on the fat-free diet showed highly increased keratinization of the epithelial layer with a pronounced thickening. The hypertrophy of the epithelial layer was characterized by an active malpighian layer. There was a hyalin-like appearance of papillae with some atrophy. The pigs treated with corn oil had a slight amount of keratinization of the epithelium at the surface and wrinkling of the skin as indicated by the papillae being closer together. The skin of the pigs receiving fat had a normal appearance. These abnormalities of the skin of the pigs on the fat-free diet are similar to those noted by Beeson and Kennelly (1947). The addition of corn oil to the diets of the fat-free pigs reduced these symptoms considerably. It may be concluded that the skin symptoms are characteristic of a fat deficiency in the pig.

There was little difference in the unsaturation of the backfats. The average Hanus iodine numbers of the fat-deficient pigs was 56 as compared to 57 for the controls. The pigs receiving 1.5% corn oil for a period of three weeks had a greater degree of unsaturation as indicated by the higher iodine number of 61. This higher number may be expected since corn oil is more unsaturated than lard; however, it is possible that this difference may be due to biological variation. The iodine numbers in this study compare favorably with those of the rat as reported by Anderson and Mendel (1928). Ellis and Isbell (1926) and Ellis and Zeller (1930) found that the deposition of fat in the hog follows that

of the rat. The synthesized depot fat of the fat-deficient pigs should be quite characteristic of the species, since it was not influenced by any exogenous source of fat.

Table 5 summarizes the analyses made of the liver. There was no significant effect of fat on the moisture or fat content of the liver. The vitamin A content of the liver was slightly higher in the pigs receiving fat than that of the pigs on the low fat diet. The addition of 1.5% corn oil to the diet of the fat-deficient pigs did not improve the vitamin A storage in the liver.

TABLE 5. ANALYSES OF THE LIVER

(Experiment II)

Items Compared	Treatment		
	Fat-Diet (5%)	Fat-Free[1] Diet (0.06%)	Fat-Free[2] With 1.5% Corn Oil
Average % of moisture	59.6	58.4	60.1
Average % of fat (on a dry weight basis)	7.1	6.8	7.5
Vitamin A (micrograms per 100 gm. liver)	1357.8	1161.4	1194.9

[1] One male and one female pig were taken from the fat-free diet at the end of the ninth week. There were only two pigs on this diet for the remaining two weeks.

[2] One male and one female pig on the fat-free diet were given 1.5% corn oil in their diet at the end of the ninth week and this treatment was continued to the end of the experiment.

Summary

1. A purified ration containing 0.12% of fat in Experiment I and 0.06% fat in Experiment II produced the following external fat deficiency symptoms: scaly dandruff-like dermatitis on the tail, back and shoulders; loss of hair; the remaining hair being dull and dry; a brown gummy exudate on the belly and sides; necrotic areas on the skin around the neck and shoulders; and an unthrifty appearance. The addition of 1.5% corn oil to the diet of the pigs receiving the fat-free ration caused some recovery of these symptoms in three weeks of treatment.

2. The pigs receiving only 0.06% ether-extractable material in their diet had a highly significantly slower growth rate than the pigs receiving 5.0% fat. Growth of these pigs tended to plateau after 63 days on the fat-free diet. The addition of 1.5% corn oil to the fat-free diet caused an immediate increase in the growth rate.

3. Feed consumption was not affected by the amount of fat present in the diet.

4. Feed efficiency was not affected by the fat content of the ration in Experiment I, but was significantly lower in the pigs receiving 0.06% fat in Experiment II. Feed efficiency increased when 1.5% corn oil was incorporated in the fat-free diet.

5. A significantly higher plasma vitamin A level was maintained in the pigs receiving the fat-free ration in both experiments.

6. Fat had no significant effect on the hemoglobin, cholesterol, red blood cell and white blood cell content of the blood.

7. Blood plasma total lipids were significantly higher in the pigs receiving 5.0% fat in their diet.

8. Pigs receiving no fat in their diet had underdeveloped digestive systems and very small gall bladders.

9. The thyroid glands of the pigs receiving 0.06% fat in their diets were enlarged.

10. Sexual maturity was retarded in the pigs receiving only 0.06% fat in their diet.

11. There was no significant difference in the saturation of the depot fat in the pigs receiving 5.0 and 0.06% fat, but the pigs that received 1.5% corn oil for 3 weeks had a slightly higher iodine number of the back fat.

12. Vitamin A storage was slightly higher in the pigs receiving 5.0% fat in their diets.

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THE EFFECT OF THYROPROTEIN ON THE GROWTH AND THYROTROPIC HORMONE CONTENT OF THE ANTERIOR PITUITARY OF SWINE[1, 2]

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THE effect of thyroidally-active substances on the growth rate of hogs has not been fully established. Braude (1947) reported seven trials with Large White Hogs to which he had given a constant level of 1.5 gm. thyroprotein per day. He observed increased rate of respiration, hyperirritability, and retarded rate of growth. Muhrer, *et al.* (1947) found no growth stimulation when pigs were fed iodinated casein for a short time during the fattening period. Vander Noot, *et al.* (1948) found that thyroprotein had little effect on growth at levels up to 0.225 gm. per 100 lb. body weight per day and that higher dosages reduced growth. However, thyroprotein feeding was discontinued when the pigs weighed 125 pounds.

In contrast, several investigators have reported a stimulation of growth rate by feeding thyroprotein to hogs. Beeson, *et al.* (1947, 1949) reported that weanling Duroc pigs receiving thyroprotein at the level of 0.0088 percent of the ration gained 26 pounds more per pig and required ten percent less feed during an 84-day feeding trial. A significant increase in body length and weight of bellies and picnic shoulders was reported. Perry, *et al.* (1948) continued this work and in a 104-day trial a practical swine ration containing 0.0132 percent thyroprotein caused a significant increase in gain of 14 pounds and also resulted in significantly longer carcasses with heavier loins. Perry, *et al.* (1950) reported significantly increased gains ($P<0.05$) of from 32 to 41 pounds with an improved feed efficiency when thyroprotein was incorporated into the ration at levels of 0.0088, 0.0132, and 0.0176 percent, respectively, for weanling Duroc pigs in dry lot.

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Wallach, *et al.* (1947) and Reineke, *et. al.* (1948) found that while eight control Durocs averaged 1.42 pounds per day gain during a seventy-nine day post-weaning period, two separate lots of seven Durocs receiving 1.4 and 2.8 gm. thyroprotein per 100 pounds feed, respectively, gained 1.54 pounds per day. Similar results were obtained by these investigators with Yorkshire, Chester White, and Berkshire pigs. Using small dosages of iodinated casein (0.005 to 0.006 percent of the ration) Reineke and McMillen (1946) reported a significant increase in the rate of gain and a slight increase in efficiency of feed utilization.

Materials and Methods

In this paper the results of three feeding trials utilizing a total of 160 Hampshire hogs are reported. The first trial was conducted on concrete floors, and the second trial on Ladino-alfalfa pasture. The third trial was started in the fall of 1949 on Ladino-alfalfa pasture, also. However, after the first two weeks a severe frost reduced the pastures to bare soil lots and thus no green material was available after the second week.

In the first trial conducted during the winter of 1948–49, fifty weanling Hampshire pigs of an average weight of 54 pounds, were separated into five lots of ten pigs on the basis of liveweight, thriftiness, and general appearance. The five lots were fed the various rations for a total of 138 days until the fastest-gaining group (Lot V) reached an average liveweight of 257 pounds.

In this trial thyroprotein was incorporated into the protein supplement and all components of the ration were fed free choice. The basal ration consisted of shelled corn, Purdue Supplement V,[4] and a mineral mixture containing equal parts of steamed bone meal, ground limestone, and salt. Lot I served as the control and received only the basal ration. Lot II received, in addition to the basal ration, 0.447 gm. of thyroprotein per 100 pounds liveweight per day with an adjustment being made each two weeks in an attempt to keep the consumption of thyroprotein at the desired level. Lot III received the basal ration plus 0.426 gm. thyroprotein per 100 pounds liveweight per day, with one adjustment of thyroprotein level in the protein supplement being made at 70 days. In addition to the basal ration, Lots IV and V received 60 and 80 grams, respectively, of thyroprotein per 100 pounds of protein concentrate,

[4] Purdue Supplement V consisted of soybean oil meal, 40; fishmeal, 20; meat scraps, 20; alfalfa meal, 10; cottonseed meal, 10 pounds.

mixed in the protein concentrate. (.289 and .395 gm. per 100 pounds liveweight per day—table 1)

At the time of slaughter the anterior hypophysis of each pig was dissected, weighed and frozen until they were assayed for thyrotropic hormone content. For assay, all the anterior lobes from the hogs of each lot were macerated together with a mortar and pestle. Two ml. of water were added to each 100 mg. of tissue so that 1 ml. of the injected solution contained 50 mg. of tissue. The methods of Bergmann

TABLE 1. THE EFFECT OF THYROPROTEIN ON GROWTH RATE, FEED CONSUMPTION, AND FEED EFFICIENCY OF SWINE

Trial I, Winter 1948–49. Fed 138 days.
(Dry-lot)

Items Compared	Lot I	Lot II	Lot III	Lot IV	Lot V
Number of pigs	10	9	10	9	10
Initial weight, lb.	54	54	56	58	54
Final weight, lb.	233	212	217	220	257
Gain per pig, lb.	179	158	161	162	203*
Daily gain per pig, lb.	1.30	1.14	1.17	1.18	1.48
Daily feed per pig, lb.	5.47	5.30	5.51	5.13	6.22
Feed per 100 pounds gain, lb.	422	464	470	434	421
Thyroprotein per day, gm.	—	.594	.583	.402	.616
Thyroprotein per 100 pounds liveweight per day, gm.	—	.447	.426	.289	.395

* Least significant difference for gain in weight at the 5% level is 23 pounds.

Treatments:
Lot I —Control—basal ration—in concrete lots.
Lot II —0.447 gm. thyroprotein per 100 pounds liveweight per day, adjusting each 2 weeks.
Lot III—0.426 gm. thyroprotein per 100 pounds liveweight per day, adjusting once.
Lot IV —60 gm. thyroprotein per 100 pounds protein concentrate.
Lot V —80 gm. thyroprotein per 100 pounds protein concentrate.

and Turner (1939), and of Elijah and Turner (1942) were followed carefully. Day-old male White Leghorn chicks were injected subcutaneously daily for four days with 0.1 ml. of the solution, containing 5 mg. of the anterior pituitary material. Thus, each chick received 0.4 ml. of a solution containing 20 mg. of the anterior pituitary tissue. The baby chicks were sacrificed on the fifth day and their thyroids were dissected and immediately weighed on a balance accurate to 0.1 mg. Each chick was inspected at post-mortem to be sure that only males were used.

In the second trial conducted during the Summer of 1949, 60 weanling Hampshire pigs averaging 79 pounds in weight were divided into five groups of twelve pigs each, and each group was placed on a one-half acre plot of Ladino clover and alfalfa pasture for a period of 98 days.

As in the first trial, all components of the basal ration were fed *ad libitum*. The basal ration consisted of—shelled corn; mineral mixture (equal parts steamed bone meal, ground limestone, and salt); and protein supplement (equal parts soybean meal and meat scraps). Lot I received only the basal ration. Lot II was fed thyroprotein at a level of 0.363 gm. per 100 pounds liveweight per day in their protein concen-

TABLE 2. THYROTROPIC HORMONE ASSAY OF ANTERIOR PITUITARIES

Trial I, Winter 1948–49. Fed 138 days.

	Lot I	Lot II	Lot III	Lot IV	Lot V
Average liveweight of pigs, lb.	233	212	217	220	257
Thyroprotein per day, gm.	—	.594	.583	.402	.616
Thyroprotein per 100 pounds liveweight per day, gm.	—	.447	.426	.289	.395
Average weights of thyroids from injected chicks, mg.	6.68	5.42	6.28	7.81	7.22

Actual Differences Between Average Thyroid Weights of test chicks:

	II	III	IV	V
	(Lot in row heading minus lot in column heading)			
I	1.26*	0.40	—1.13*	—0.54
II		—0.86	—2.39**	—1.80**
III			—1.53**	—0.94
IV				0.59

* Difference significant at 5% level.
** Difference significant at 1% level.

trate, with one adjustment being made midway during the trial. Lot III was handled similarly to Lot II except that the level of thyroprotein in this lot was 0.644 gm. per 100 pounds liveweight per day. Lot IV received the basal ration only until the animals reached an average liveweight of 136 pounds at which time the feeding of thyroprotein at a level of 0.579 gm. per 100 pounds liveweight was started. Lot V received the basal ration plus 120 gm. thyroprotein per 100 pounds protein concentrate. No carcass studies were made in this trial.

In the third trial, conducted during the winter of 1949–50, 60 weanling Hampshire pigs of an average weight of 74 pounds were divided into five lots of twelve pigs each. The original plan of this trial was to conduct it on fall pasture with each lot being assigned to a one-half acre plot. After the second week, however, a killing frost reduced the pastures to barren lots and no green material was available for the remainder of the trial.

Actually, this trial consisted of two separate comparisons. In one phase thyroprotein was fed as a part of a single complete ration, while

in the other phase thyroprotein was incorporated in the protein supplement and all components of the ration were fed free choice. Lot I served as the control for one comparison and received only the basal ration of a single complete feed which contained Purdue Supplement V, ground corn, ground wheat and minerals. Lot II received the basal ration listed for Lot I with the exception that thyroprotein was incorporated into the ration at the level of 8 gm. per 100 pounds of feed (0.0176%). Lot III served as a second control and received an *ad libitum* ration of ground shelled corn and wheat (7 to 2), Purdue Supplement V, and minerals. Lot IV received the same ration as Lot III with the exception that thyroprotein was incorporated into the supplement so that the animals received 0.487 gm. thyroprotein per 100 pounds liveweight per day with adjustments being made each two weeks. Lot V received the ration listed for Lot III plus thyroprotein adjusted every two weeks so that the ration contained 0.500 gm. thyroprotein per 100 pounds liveweight per day up to the time the animals weighed 136 pounds; then the level was dropped to 0.400 gm. until the animals weighed 180 pounds; from that time on, the level of thyroprotein was dropped to 0.300 gm. per 100 pounds liveweight per day, giving an overall average of 0.341 gm. per 100 pounds liveweight per day.

Results

Influence of Thyroprotein on Growth and Feed Consumption

Trial I—(Winter—1948–49). As summarized in table 1, with the exception of Lot V which received thyroprotein at the rate of 80 grams per 100 pounds protein supplement, none of the thyroprotein-fed pigs gained so rapidly as the controls. The control animals finished the 138-day feeding trial at an average weight of 233 pounds while the average final weight of the animals in Lot V was 257 pounds. The increased gain of 24 pounds per pig made by Lot V over Lot I was significant ($P<0.05$). Thyroprotein consumption did not result in greater feed consumption whereas in the case of Lots II and IV it resulted in reduced feed consumption.

Trial II—(Summer—1949). The feeding of thyroprotein on pasture at the level of 0.363 gm. (Lot II) or at the level of 0.442 gm. (Lot V) per 100 pounds liveweight per day to weanling Hampshire pigs did not alter growth rate significantly. When thyroprotein was fed at the level of 0.579 gm. per 100 pounds liveweight per day to pigs starting at a liveweight of 139 pounds (Lot IV) a depression in growth rate was noted. Significantly depressed growth rate ($P<0.05$) resulted when

thyroprotein was fed at a level of 0.644 gm. per 100 pounds liveweight per day. Thyroprotein-fed animals required from 5 to 20 percent more feed than the control animals per unit of gain (table 3).

Trial III—(Winter—1949–50). Thyroprotein fed in a complete ration at a level of 0.0176 percent of the ration (Lot II) to weanling Hampshire pigs resulted in slight, but not significantly, increased growth rate. The feeding of thyroprotein at a constant level of 0.487 gm. per 100 pounds liveweight per day (Lot IV) had no effect on growth rate whereas the feeding of thyroprotein in decreasing levels as the animal

TABLE 3. THE EFFECT OF THYROPROTEIN ON THE GROWTH RATE, FEED CONSUMPTION AND FEED EFFICIENCY OF SWINE

Trial II, Summer 1949. Fed 98 days.
(Pasture)

Items Compared	Lot I	Lot II	Lot III	Lot IV	Lot V
Number of pigs	12	12	12	12	12
Initial weight, lb.	76	79	77	81	80
Final weight, lb.	230	233	196	220	225
Gain per pig, lb.	154	154	119*	139	145
Daily gain per pig, lb.	1.56	1.57	1.22	1.41	1.48
Daily feed consumed, lb.	5.94	6.22	5.55	6.22	6.41
Feed per 100 pounds gain, lb.	380	396	455	439	434
Thyroprotein per day, gm.	—	.567	.884	1.036	.677
Thyroprotein per 100 pounds liveweight per day, gm.	—	.363	.644	.579	.442

* Least significant difference for gain in weight at the 5% level is 30 pounds.

Treatments:
Lot I —Control—basal ration—on pasture.
Lot II —0.363 gm. thyroprotein per 100 pounds liveweight per day.
Lot III—0.644 gm. thyroprotein per 100 pounds liveweight per day.
Lot IV —Starting at 139 pounds, 0.579 gm. thyroprotein per 100 pounds liveweight per day.
Lot V —120 gm. thyroprotein per 100 lb. protein supplement.

became heavier (Lot V) and at an overall average of 0.341 gm. per 100 pounds liveweight per day resulted in a significantly increased growth rate of 17 pounds more ($P<0.05$) than the controls, Lot III (table 4).

Influence of Thyroprotein on the Thyrotropic Hormone Content of the Anterior Hypophysis

The activity of the thyroid gland in the normal animal is regulated by the thyrotropic hormone of the anterior pituitary gland. It is known that there are species, age, sex, seasonal, breed, and strain differences in thyrotropic hormone production and that the level of secretion can be altered by numerous environmental factors such as temperature, iodine,

and goitrogens. When exogenous thyroxine or thyroidally-active substances are administered the thyroid gland ordinarily reduces its activity and the production of the anterior pituitary thyrotropic hormone may be affected.

In an attempt to study some of the above effects, an assay of the thyrotropic hormone content of the anterior pituitary glands of the swine in this trial was made. (The procedure used was that outlined by Elijah and Turner (1942)). Day-old Leghorn cockerels were

TABLE 4. THE EFFECT OF THYROPROTEIN ON GROWTH RATE, FEED CONSUMPTION, AND FEED EFFICIENCY OF SWINE

Trial III, Winter 1949–50. Fed 98 days.
(Fall Pasture)

Items Compared	Lot I	Lot II	Lot III	Lot IV	Lot V
Number of pigs	12	12	12	12	12
Initial weight, lb.	74	74	74	74	74
Final weight, lb.	224	229	216	213	233
Gain per pig, lb.	150	155	142	139	159*
Daily gain per pig, lb.	1.53	1.59	1.45	1.42	1.63
Daily feed per pig, lb.	7.25	7.10	6.22	7.40	7.60
Feed per 100 pounds gain, lb.	473	447	429	521	467
Thyroprotein per day, gm.	—	.568	—	.677	.525
Thyroprotein per 100 pounds liveweight per day, gm.	—	.374	—	.487	.341

* Least significant difference for gain in weight at the 5% level is 16 pounds.

Treatments:
Lot I —First control—one complete ration.
Lot II —Basal ration number 1 plus 0.0176% thyroprotein.
Lot III—Second control—components of ration fed free choice.
Lot IV —Basal ration number 2 plus 0.487 gm. thyroprotein per 100 pounds liveweight per day.
Lot V —Basal ration number 2 plus an average of 0.341 gm. thyroprotein per 100 pounds liveweight per day. (Decreasing levels.)

injected with suspensions of pituitary materials from the five lots of swine. An assay showed highly significant differences in thyrotropic hormone content between lots of animals which had been subjected to the differing treatments.

The anterior pituitaries of the animals which received the largest amounts of thyroprotein per 100 lb. liveweight—Lots II and III—contained the smallest amounts of thyrotropin. The pigs in Lots IV and V, which received an average of 0.289 and 0.395 grams of thyroprotein per 100 pounds of liveweight had anterior pituitaries with the largest amounts of thyrotropin (table 2).

Discussion

In all of the thyroprotein research with swine conducted at the Indiana Station prior to these trials, thyroprotein was blended into the ration as a definite percentage of the ration, i.e., 0.0044, 0.0088, 0.0132, or 0.0176 percent. Since the growing pig consumes decreasing amounts of feed, in proportion to body weight, as it increases in size, it follows that where thyroprotein comprises a definite percent of the ration, the animals consume less per unit of weight with increasing size. In the trials reported in this paper some of the levels of thyroprotein did not decrease, in proportion to liveweight, as the pigs grew larger. In these trials it was observed that the only instances where thyroprotein-fed pigs significantly outgained their controls was when the level of thyroprotein decreased in proportion to liveweight as the pigs grew in size. This agrees with the data reported by Reineke *et al.* (1948) with pigs, and Hurst and Turner (1947) with mice, in which they indicate that it is necessary to reduce the thyroprotein dosage per unit body weight as growth progresses if growth stimulation is to be maintained. Braude (1947) administered thyroprotein to growing pigs at constant levels, regardless of body weight, and reported that lower levels were of no effect whereas higher levels affected the animals adversely.

Vander Noot *et al.* (1948) were unable to show a growth response by the feeding of 0.075 to 0.225 gm. thyroprotein per 100 pounds body weight per day. Higher levels of 0.5 to 4.0 gm. per 100 pounds body weight resulted in growth depression. One feature of this trial that may partially explain the results was that the animals were fed to a liveweight of but 125 pounds when the trial was concluded. In all of the work with swine at the Indiana Station it has been observed that very little difference in growth rate was noticeable between animals receiving thyroprotein and the controls until the animals reached an average liveweight of 130 pounds. Another point concerning the New Jersey work was that the level of thyroprotein per unit of liveweight remained constant and did not decrease as the animals became larger.

Thyrotropic hormone content of the anterior pituitary glands from the hogs of the Winter Trial of 1948–1949 was assayed by the chick assay method outlined by Elijah and Turner (1942). The general trend of the analysis indicated that the faster growing hogs had anterior pituitaries with higher levels of thyrotropic hormone. The level of thyrotropic hormone in the various anterior pituitary glands bore a relation to the level of consumption of thyroprotein. Animals which consumed the highest levels of thyroprotein, per unit of body weight, had glands with the lowest assays of thyrotropic hormone.

Summary

In the trials reported in this paper the only instances in which the feeding of thyroprotein resulted in an increased growth rate in swine were those in which the level of thyroprotein fed per unit of body weight decreased as the animals grew larger. (Trial I, Lot V; Trial III, Lots II and V).

The feeding of thyroprotein to hogs on pasture in no case stimulated growth rate and in one instance (Trial II, Lot III) depressed growth rate significantly.

Animals which consumed the highest levels of thyroprotein, per unit of body weight, had anterior hypophyses with the lowest assays of thyrotropic hormone.

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MINIMAL VOLUME OF SEMEN AND NUMBER OF SPERM FOR FERTILITY IN ARTIFICIAL INSEMINATION OF SWINE[1]

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FEW studies of artificial insemination in swine have been made. Rodin and Lipatov (1935) reported from one experiment that the optimum dilution rate of boar semen was 1 part semen to 4 parts diluter and the optimum volume of diluted semen was 100 to 150 cc. depending on the size of the sow. The fertility in sows inseminated according to these standards was 70%. In another study where quantities of undiluted semen ranging from 1 to 100 cc. were used, these workers found that fertility was highest when inseminations were made on the second day of heat. Lambert and McKenzie (1940) state that 50 to 100 cc. of undiluted semen seem sufficient if sperm concentration is reasonably high but the basis for these recommendations is not given. Lasley (1940) found that 53% of 38 gilts settled when inseminated twice during the heat period with semen stored for 24 hours while only 23% of 30 gilts settled when inseminated once during estrus with semen stored for 24 hours.

From these studies it appears that artificial insemination of swine might be practical under certain conditions. Especially would this be true if the volume of semen needed for fertility can be reduced so that more females can be inseminated with a single ejaculate from a boar. This study was made to determine the minimal volume of semen and minimal number of sperm necessary for fertility in artificial insemination of sows and gilts.

Materials and Methods

Most of the gilts in this study were purchased from a local packing company and their color markings indicated that they were crossbred. The remainder of the gilts were outbred Chester Whites and Poland Chinas from the University of Wisconsin herd. Most of the sows were inbred Chester Whites and inbred Yorkshires and the remainder were grade Chester Whites and Poland Chinas. Twelve boars ranging from

[1] Paper No. 440 from the Department of Genetics, University of Wisconsin.

8 months to 4 years of age and of the following breeding were used: outbred Chester White, outbred Poland China, inbred Chester White, inbred Yorkshire, and an inbred strain originating from a cross of Chester White x Yorkshire.

The females were checked for heat twice daily with vasectomized boars and inseminated at the second heat check, i.e. 12 to 23 hours after the onset of estrus. The quantity of fresh semen to be used was diluted to a standard volume of 50 cc. (the minimum volume of undiluted semen recommended by Lambert and McKenzie, 1940) in a modified Krebs solution (Lardy and Phillips, 1942) in all gilts. Two volumes, 50 and 250 cc. were used in sows. Semen was collected by artificial vagina and strained through cheesecloth to remove the gelatinous fraction. The semen was then examined for the presence of motile sperm (samples containing less than 10% sperm showing forward motility were discarded) and used as soon as possible, usually within 15 minutes after collection. A single ejaculate was used to inseminate 2 animals in only 2 cases; in all other instances where 2 or more animals were to be inseminated at the same time a separate ejaculate was used for each insemination. Sperm counts of all ejaculates were made with a hemocytometer.

A semi-flexible plastic tube, ¼ inch outside diameter and ⅛ inch inside diameter, attached by means of a short length of rubber tubing to a 50 cc. glass syringe was used for the insemination. Semen was deposited in the cervix in all gilts and most sows but in a few sows semen was deposited in the uterus.

All animals were killed 24 to 48 hours after the end of heat and the eggs were recovered and examined to determine the fertilization rate.

Results and Discussion

Fertility in Gilts

Gilts were inseminated with quantities of fresh semen varying from 0.01 to 20.0 cc. and differences in fertility with different volumes of semen were significant. The smallest volume of semen which resulted in fertility (equally cleaving eggs 1 to 2 days after the end of heat) was 0.1 cc. Only 2 animals were inseminated with less than 0.1 cc., however, and it is possible that some fertility would have been obtained with smaller volumes had more animals been used. When 0.1 cc. of semen was used none of the animals had all fertilized eggs, 2 animals had no fertilized eggs and the average fertility (unweighted mean) was 29%

(table 1). The average fertility was 40% when 1 cc. of semen was used and 51% when 5 cc. was used but there was no further increase in fertility (actually a 2% decrease) when 10 cc. was used. There was a decided tendency, especially at the 5 and 10 cc. levels for the gilts to have either complete fertility or no fertility at all with very few individual animals having a percentage of fertilized eggs that corresponded even roughly to the average fertility for their group.

There was a marked increase in fertility when 20 cc. of semen was used. At this level 8 of the 10 animals had all fertilized eggs and the other 2 had some fertilized eggs. The average fertility per gilt was 91%

TABLE 1. FERTILITY OBTAINED WITH DIFFERENT VOLUMES OF SEMEN IN ARTIFICIAL INSEMINATION OF SOWS AND GILTS

Volume of Fresh semen, cc.	Average Number of Sperm (000)	Total Volume, cc.	Number of Animals	Number of animals having percentage of fertilized eggs of:						Average Percent Fertility
				0	1–24	25–49	50–74	75–99	100	
			Gilts							
0.01	1,590	50	2	2	—	—	—	—	—	0
0.1	20,267	50	6	2	1	2	—	1	—	29
1	187,600	50	10	4	—	1	3	1	1	40
5	580,667	50	9	2	2	—	—	3	2	51
10	1,141,111	50	9	4	—	—	1	1	3	49
20	3,504,800	50	10	—	—	1	—	1	8	91
			Sows							
1	234,500	50	10	3	1	2	1	2	1	42
20	4,688,000	50	9	4	1	—	—	2	2	43
20	2,860,000	250	9	3	2	—	1	—	3	43
50	6,625,000	50	6	5	—	1	—	—	—	4
50	5,931,667	250	6	0	2	1	—	1	2	57

which compares favorably with the 82% resulting from natural service in this laboratory (Warnick, *et al.*, in press) and the 93% reported by Squires, *et al.* (1949).

Fertility in Sows

The differences in percentages of fertilized eggs with different volumes of semen were not significant in sows. The results obtained with 1 cc. of semen in sows were very similar to those obtained with the same quantity of semen in gilts. However, in sows, the average fertility per animal was as high with 1 cc. of semen diluted to a total volume of 50 cc. as it was with 20 cc. diluted to either 50 or 250 cc. and actually higher than the average fertility in 6 sows inseminated with 50 cc. of undiluted semen. At these levels there was a tendency for individual animals either to have all eggs fertilized or to have no eggs fertilized.

Some fertility was obtained in all sows inseminated with 50 cc. of semen diluted to a total volume of 250 cc. and the average fertility for the group was 57%. Thus it would appear that both a greater number of sperm and a greater volume of semen are required for good fertility in sows than are required in gilts when both are inseminated 12 to 23 hours after the onset of estrus. The sows did not have appreciably longer heat periods than the gilts (table 2) so a longer period of time from insemination to ovulation in sows would not appear to be a factor. The sows did, however, have longer uterine horns and it is possible that sows require a larger volume of semen (and more sperm?) to have an effective number of sperm transported to the site of fertilization. Unfortunately no data are available on fertilization rates resulting from

TABLE 2. COMPARISON OF OVULATION RATE, LENGTH OF HEAT PERIOD AND LENGTH OF UTERINE HORNS IN SOWS AND GILTS

Item	Sows	Gilts
Number of animals	40	46
Ovulation rate	17.8	13.0
Length of heat period (Number of 12-hour checks "in heat")	4.2	4.0
Uterine horn length—inches	42.9	31.1

TABLE 3. EFFECT OF PARTIAL FERTILITY ON LITTER SIZE IN SOWS AND GILTS

Volume of Fresh semen, cc.	Total Volume, cc.	Average percent Fertility in Animals Having some cleaved eggs	Estimated Maximum Avg. Litter size[1]
		Gilts	
0.1	50	44	5.7
1	50	67	8.7
5	50	65	8.5
10	50	89	11.6
20	50	91	11.8
		Sows	
1	50	60	10.7
20	50	75	13.4
20	250	64	11.4
50	50	25	4.5
50	250	57	10.1

[1] Based on ovulation rates of 13.0 in gilts and 17.8 in sows.

natural service in normal sows so it is not known how the fertility of 57% obtained with 50 cc. of fresh semen diluted to a total volume of 250 cc. compares with the fertility to be expected in sows from natural service.

Effect of partial fertility on expected litter size

Those animals that had some fertilized eggs were used to calculate the potential effect of partial fertility on litter size. The maximum-possible average litter sizes in gilts (based on an average ovulation rate of 13.0 eggs) ranged from 5.7 to 11.8 pigs with the different volumes of semen (table 3). The maximum average litter sizes in sows (based on an ovulation rate of 17.8 eggs) ranged from 4.5 to 13.4 pigs per litter. Since Hauser, *et al.* (1949) has reported that the sperm concentration is low in the semen of young boars shortly after puberty and Rodolpho (1935) and McKenzie, Miller and Bauguess (1938) have reported that semen volume and sperm concentration are reduced in mature boars subjected to a heavy mating schedule it would appear that under these conditions it would be possible for a boar to affect the sizes of the litters farrowed by his mates. Any other situation which would reduce the number of sperm per ejaculate to the levels used in this experiment would also be expected to have an effect on litter size.

Discussion

The problems of diluters, temperatures, etc. involved in storing boar semen for appreciable lengths of time must be solved before artificial insemination can have a widespread practical application in swine but these results indicate that in special situations artificial insemination, particularly of gilts, could be used to advantage. Some such cases would be the breeding of several gilts to the same boar with one ejaculate and the breeding of gilts to extremely heavy boars or to boars where natural service is undesirable because of disease problems. Unless the quantity of fresh semen required for fertility can be lowered appreciably from the levels found in this experiment artificial insemination of sows would appear to have less potential value.

From an experimental standpoint artificial insemination of both sows and gilts might be employed with "optimal" numbers of sperm to obtain a critical level at which to study the effect of various factors on fertility since "treatment differences" could probably be detected more easily in cases where fertility was already borderline.

Summary

Forty-six gilts were inseminated with quantities of fresh semen ranging from 0.01 to 20 cc. diluted to a total volume of 50 cc. in a modified Krebs' solution and 40 sows were inseminated with quantities of 1 to 50 cc. of fresh semen diluted to either 50 or 250 cc. In gilts some fertility (29%) was obtained with 0.1 cc. semen and an average fertility of 91% was obtained with 20 cc. semen diluted to 50 cc. In sows fertility was almost as high (42%) with 1 cc. of semen diluted to 50 cc. as it was with 20 cc. of semen diluted to either 50 or 250 cc. (43% in each case). The average fertility per sow was 67% when 50 cc. of semen diluted to 250 cc. was used.

Maximum-possible average litter sizes were estimated at 5.7 to 11.8 pigs in gilts and from 4.5 to 13.4 pigs in sows inseminated with different quantities of semen. Sows ovulated 4.6 more eggs, had 11.8 inch longer uterine horns and stayed in heat slightly longer than gilts.

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APF SUPPLEMENTS IN MILK REPLACEMENTS FOR DAIRY CALVES[1, 2]

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CONSIDERABLE research has been conducted upon the inclusion of the Animal Protein Factor and some of its components in the rations of swine and poultry. Using a low protein calf starter without animal protein, Cunningham and Nelson (1944) obtained normal growth in dairy calves weaned at 28 days of age. Cunha *et al.* (1949) reported an APF supplement in either a corn-peanut or corn-soybean ration produced better growth in swine than did corn-peanut or corn-soybean basal ration. Anderson and Hogan (1949) using weanling pigs, showed a vitamin B-12 concentrate or fish meal when added to a basal corn-soybean ration produced larger gains than did the basal ration. Hale and Lyman (1949) fed a vitamin B-12 concentrate to growing pigs and noted 31 percent better gains than the controls. On the other hand, Colby and Ensminger (1950) noted there was no advantage in adding vitamin B-12 concentrate, injected crystalline vitamin B-12, an APF concentrate, or alfalfa meal and fish meal to the ration of the weanling pig on a purified basal diet.

Lepley, Catron and Culbertson (1949) have determined the vitamin B-12 requirement of growing-fattening pigs to be between 5 and 10 micrograms per pound of feed. Newman *et al.* (1950) have found the oral requirement of vitamin B-12 in the diet of the baby pig to be approximately 50 gamma per kilo of dry matter in the ration. Ott, Rickes and Wood (1948) determined the optimal growth requirement of vitamin B-12 for day-old chicks to be less than 30 gamma per kilo of diet. Stokstad *et al.* (1949) have stated that 1.5 gamma per kilo of diet or 0.3 gamma injected intramuscularly each week supplied the vitamin B-12 requirement of chicks but other supplements had to be added for maximum effect.

Reece (1950) found that a heifer on a dried milk solids, vitamin supplemented diet and fed 50 gamma of vitamin B-12 daily gained no

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[2] The data contained in this publication are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

better than did a control on the same diet. Rusoff and Haq (1950) did not receive additional growth in calves weaned from milk at 28 days of age when 5 gamma per pound of feed were added to a basal vegetable protein calf starter.

An experiment was conducted to study the effect of adding APF concentrates to a milk replacement diet for dairy calves.

Experimental Procedure

Fifteen male Holstein calves, seven days of age, were divided into three groups of five calves each on the basis of body weight, withers height and chest circumference. These calves were obtained from state institutional herds that were fed a ration of corn silage, fair grade of mixed hay and 16 percent total protein grain mixture.

The calves were housed in individual solid-wall pens equipped with a water bowl, salt block, hay rack and concentrate box located 14

TABLE 1. FEEDING SCHEDULE

Days of Age	Whole Milk lb.	Milk Rep. lb.	Warm Water lb.
First 7 days—Dam's Colostrum and Whole Milk			
8–14	2	0.2	2
15–21	0	0.3	4
22–28	0	0.4	4
29–42	0	0.5	5
43–49	0	0.6	6

inches from the floor. The stable temperature was maintained at 65° F. in an artificially lighted and ventilated barn by means of thermostatically controlled steam heat and exhaust fans.

The calves were fed the milk replacement rations, which has been successful with over one-thousand calves, from open pails (table 2) at 100° F. twice daily according to schedule (table 1).

The ingredients in the milk replacement formula were ground very fine by means of a hammer mill and a thousand pound batch mixed by hand. The mix was then divided into three equal lots and the supplements added as indicated (table 2).

A calf starter which has been developed and proven successful (table 3) was fed *ad libitum* until the maximum daily allowance of 6 pounds was reached. A good quality grass-legume hay was fed *ad libitum*. Each calf had access to water, calf starter and hay from the eighth day of age until the conclusion of the 49 day trial. Measures of body growth

TABLE 2. MILK-REPLACEMENT FORMULAE

Ingredient	Formulae		
	I	II	III
Dried skimmilk	50 lb.	50 lb.	50 lb.
Dried Whey	10	10	10
Distillers Corn Solubles	10	10	10
Blood Flour	10	10	10
Oat Flour	5	5	5
Cerelose	7.75	7.75	7.75
Brewers Yeast	4.90	4.90	4.90
Irradiated Yeast (9F)	0.10	0.10	0.10
Stabilized Vitamin A Feed[1]	0.22	0.22	0.22
Mineral Mixture[2]	0.042	0.042	0.042
Dicalcium Phosphate	2.50	2.50	2.50
APF Supplement[3]		2.2	
APF Supplement[4]			0.3

[1] The supplement contained 2,220,000 USP Units per pound.
[2] Mineral Mixture:

Ferric Citrate ($FeC_6H_5O_7 \cdot 3H_2O$)	56.57%
Cupric Sulfate ($CuSO_4 \cdot 5H_2O$)	19.73
Manganese Sulfate ($MnSO_4 \cdot 4H_2O$)	21.59
Cobalt Chloride ($CoCl_2 \cdot 6H_2O$)	2.11

[3] Three to four micrograms per gram B-12 equivalent.
[4] 27.5 micrograms per gram B-12 equivalent.

TABLE 3. STARTER FORMULA

Ingredient	Lbs.
Ground yellow corn meal	416.5
Wheat bran	300.0
Crimped whole oats	400.0
Linseed oil meal	100.0
Soybean oil meal (44%)	300.0
Dehydrated alfalfa meal	150.0
Cane molasses	100.0
Dried skimmilk	100.0
Distillers dried corn solubles	100.0
Irradiated yeast (9F)	0.5
Dicalcium phosphate	10.0
Ground limestone	10.0
Iodized salt	10.0
Vitamin A meal[1]	3.0

[1] 2,270,000 USP Units per pound in dry meal form.

were taken at weekly intervals and feces observations made daily by the same person.

Results and Discussion

Analysis of variance of growth measurements did not reveal a significant difference between groups (table 4). Increased consumption

of calf starter was evident in the later days of the experiment. The authors are aware that ration II contained small amounts of aueromycin and that ration III contained some streptomycin. It is conceivable that there was enough of the antibiotics in the supplemented rations to affect the desirable flora in the digestive tract of the calf and thus decrease efficiency of feed utilization. The beginning consumption of calf starter was comparable in all groups and there were no differences in hay consumption between groups.

TABLE 4. MEAN DAILY GAIN AND MEAN CONCENTRATE CONSUMPTION FROM BIRTH TO 49 DAYS OF AGE

Group	Mean Daily Gain			Mean Starter Consumption Lb.
	Body Weight Lb.	Withers Height Cm.	Chest Circ. In.	
Control I	0.92	0.12	.08	54
APF II	0.82	0.12	.08	72
APF III	0.92	0.12	.08	65

The milk-replacement feeding level was kept particularly low in order to measure more accurately any added increase in growth due to the APF supplementation. Microbiological analysis of the basal ration revealed the vitamin B-12 equivalent to be approximately 2 gamma per pound of feed. The vitamin B-12 equivalent of the two supplemented rations was between 35 and 40 gamma per pound of feed. On this basis the calves on the basal ration received 0.8 gamma of vitamin B-12 equivalent on the eighth day of age and calves in the supplemented groups received 14 to 16 gamma.

There were no fatalities in any of the groups and it was not necessary to administer antibiotics to control diarrhea or other abnormal conditions. Palatability of the milk-replacement-water mixture was excellent and the general well-being of all of the animals was very satisfactory.

Summary

Fifteen male Holstein calves, 7 days of age, were divided into 3 comparable groups. One group was fed the basal ration and the other two groups were fed the basal ration supplemented with APF concentrates.

Under the conditions of this experiment, the addition of APF supplements to the basal milk replacement ration used, did not enhance growth above that obtained from the basal group.

The average calf starter consumption was higher for the supplemented groups than for the basal group.

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THE BASAL ENERGY AND NITROGEN METABOLISM OF DAIRY CALVES IN HYPERTHYROIDISM INDUCED BY THYROPROTEIN ADMINISTRATION

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THE role of thyroidal materials in animal production has been reviewed recently by Blaxter *et al.* (1949). The value of these materials in increasing the milk production of dairy cattle and other types of animal production has been appraised critically by these reviewers, who conclude that the control of thyroid function at will by the use of such materials "not only gives promise of some applications of economic importance, but also provides a research tool that could help to advance a step farther the frontiers of our knowledge of the physiologic processes upon which livestock production is based." A natural extension of this problem is a study of the metabolic cost of the increased production secured by administration of thyroid-active materials.

Observations of the physiology of the hyperthyroid ruminant (Blaxter, 1948a, 1948b; Owen, 1948a, and Reece, 1950), and of its metabolism of energy (Thorbek, Hansen and Moustgaard, 1948) of nitrogen (Blaxter, 1948a; Owen, 1948a) and of minerals (Owen, 1948b; Blaxter, 1948a), reveal the profound effect of thyroid stimulation on the tissues of the animal. The increase in the milk and fat production of the dairy cow under conditions of induced hyperthyroidism is apparently merely one phase of the general metabolic stimulus mediated by the thyroid gland. The increased nutrient requirements of the animal resulting from this metabolic stimulation can be covered by an increased food intake, but the stimulus to milk and milk-fat production occurs whether or not the food supply is raised.

The well-established stimulating effect of thyroxine on the basal metabolism of animals has been confirmed by Blaxter (1948c) on sheep using iodinated casein as the thyroid active material. Blaxter found that the effect of this product on the basal metabolism was linearly related to the dosage expressed in mg. per kg. $W^{.73}$. In another publication (1948a), Blaxter estimates the endogenous nitrogen catabolism of sheep receiving different dosages of iodinated casein, on the assumption

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that the biological value of the ingested nitrogen is unaffected by hyperthyroidism. The endogenous nitrogen excretion, so computed, was found to increase in a linear fashion with dosage.

The excess energy and nitrogen expended in hyperthyroidism is a measure, in these terms, of the cost of any increased production of milk or milk fat that may result in the dairy cow. It was the purpose of the experiments to be reported below to examine these increased expenditures in the young bovine, determining directly both the basal-metabolism and the endogenous nitrogen output in the normal and in the hyperthyroid condition. A point of equal interest was the determination of the effect of hyperthyroidism on the ratio of endogenous nitrogen to basal calories, a ratio that, under usual conditions, is remarkably constant for different species of animals (Smuts, 1935), although affected by age and environmental temperature, in the rat at least (Treichler and Mitchell, 1941). In a previous publication (1949) the authors have shown that, in the rat, a degree of hyperthyroidism, induced by the administration of iodinated casein, sufficient to raise the basal metabolism 80 to 90 percent, had no appreciable effect on the ratio of endogenous nitrogen output to basal heat. The experiments reported in this paper reveal a somewhat different situation for the young bovine.

Experimental Methods

The subjects of the experiments were two Holstein bull calves weighing initially 270 and 340 lbs. The schedule of the experiments, together with daily food intakes per 100 lbs. body weight and average daily change in body weight per period, is given in table 1. The experimental rations are described in table 2. In the hyperthyroid periods (VIII to XII), the iodinated casein, protamone, was administered every morning in gelatin capsules.

The dosage of protamone given was computed from the equation: $P=200\ W^{0.73}$, in which P is the dosage in mg. and W is the body weight in Kg. This dosage of protamone would be expected to raise the basal metabolism of the animals by about 25 percent according to observations made on rabbits in this laboratory by Blaxter (personal communication). During the hyperthyroid periods, the calves were confined in our air-conditioned chamber maintained at 82° F.

The Basal Metabolism

The basal metabolism of the calves was determined in a pneumatic chamber of known volume, in which the air was circulated by two

TABLE 1. SCHEDULE OF EXPERIMENTS, WITH FOOD INTAKE AND BODY WEIGHT CHANGE OF CALVES

Period No.	Ration	Average body wts.		Length of period	Calf. No. 60		Calf No. 965		Determinations Made
		Calf 60 lbs.	Calf 965 lbs.		Daily Food intake per 100 lbs. body wt. lbs.	Gain in body wt. per day lbs.	Daily Food intake per 100 lbs. body wt. lbs.	Gain in body wt. per day lbs.	
					Normal Thyroid Period				
I	Standard	333	268	2 weeks	1.4	±0.0	1.8	−1.3	.....
II	Fasting	321	240	72 hours	none	.....	none	.....	B.M.R.
III	Adjustment to "low-N"	341	264	5 days	1.4	±0.0	1.9	±0.0	.....
IV	"Low-N"	334	260	6 days	1.5	−1.0	2.2	±0.0	Urinary N and creatinine
V	"N-free"	316	248	12 days	1.5	−1.8	2.5	−1.4	Urinary N and creatinine
VI	Standard	321	244	2 weeks	1.8	+2.0	1.9	+1.0	.....
VII	Fasting	312	236	72–90 hrs.	none	.....	none	.....	B.M.R.
VII(a)	Standard	320	240	2 weeks	2.2	±0.0	1.9	±0.0	.....
					Hyperthyroid[1] Period				
VIII	Standard	318	245	2 weeks[2]	2.2	−0.6	2.1	±0.0	.....
IX	Fasting	283	235	72–96 hrs.	none	.....	none	.....	B.M.R.
X	Adjustment to "low-N"	266	237	8 days	2.4	−3.0	2.4	±0.0	.....
XI	"Low-N"	255	231	11 days	2.8	−1.0	2.5	±0.0	Urinary N and creatinine
XII	"N-free"	248	217	6–9 days	3.0	±0.0	3.4	−2.6	Urinary N and creatinine

[1] Hyperthyroidism was induced by feeding "protamone" (iodinated casein), obtained from the Cerophyl Laboratories, Inc., Kansas City, Missouri, through the courtesy of Dr. W. R. Graham, Jr.

[2] Four weeks in case of calf 60.

electric fans and the humidity minimized by blowing the air over P_2O_5. The air in the chamber was sampled at the beginning of a determination and again at the end of 2.5 to 3 hours, and the samples were analyzed for CO_2 and O_2. The quantity of CH_4 produced after a fast of 72 hours was negligible. The computation of heat production was made on the basis of the respiratory exchange, with necessary corrections for any inward leakage of outside air that may have occurred as indicated by the nitrogen content of the chamber. The pressure in the chamber was maintained slightly less than atmospheric, and the inside temperature

TABLE 2. COMPOSITION OF EXPERIMENTAL RATIONS

1. Standard ration [12% (N x 6.25)]	
Oat straw	1 part
Dairy mixture[1]	1 part
Wheat mixture[2]	½ part
2. "Low-N" ration [1% (N x 6.25)]	
Oat straw	5 parts
"N-free" mixture	17 parts
Bone meal	½ part
Oil mixture[3]	½ part
3. "N-free" ration [0.25% (N x 6.25)]	
"N-free" mixture	33 parts
Oil mixture	1 part
4 "N-free" mixture	
Corn starch	20 parts
Brown sugar	6 parts
Dextrinized starch	5 parts
Wood flock	5 parts
Salt mixture (no. 446)[4]	1.2 parts

[1] The dairy mixture consisted of crushed oats, 30 parts; ground corn, 25 parts; linseed meal, 15 parts; alfalfa lead meal, 10 parts; dried skim milk, 10 parts; soy bean meal, 6 parts; wheat bran, 2 parts; salt, 1 part; powdered limestone, 0.5 part; steamed bone meal, 0.5 part; and dried yeast, 0.05 part.

[2] The wheat mixture contained ground wheat, 99 parts, and salt, 1 part.

[3] The oil mixture was composed of corn oil, 8 parts, and cod liver oil, 1 part.

[4] The composition of the salt mixture is given by Spector (1948).

was kept within a comfortable range (75 to 85° F.) by running a stream of cold water over the top of the chamber.

The difficulty of maintaining farm animals in complete muscular repose has always been a handicap in the measurement of their basal metabolism. The successful use of nembutal anesthesia by Reineke *et al.* (1941) in milk secretion studies with goats suggested the possibility of using this drug in securing valid measurements of the basal metabolism of the experimental calves. The nembutalized goats of Reineke and colleagues secreted milk of normal composition at a normal rate. Hypnotic drugs administered in doses just sufficient for narcosis over a period adequate for metabolism measurement have not been found to depress the basal metabolism of animals considerably (Boothby and Rowntree, 1923–24; Siebert and Thurston, 1931–32), perhaps not more than corresponds to the greater muscular quiescence induced, but

larger doses, producing deep narcosis and bordering on lethal doses, may depress basal metabolism, as ordinarily determined, by as much as 20 percent. (Lee, 1928; Brody *et al.*, 1932).

Nembutal (pentobarbital sodium) appeared to be the narcotic of choice, but before deciding to use it on the experimental calves, preliminary tests were made with a ewe lamb weighing about 66 lbs. In these experiments, the expired gases were collected, after a 72 to 78 hour fast, in a Collins gasometer, employing a rubber mask fitted snugly over the muzzle of the ewe, the muzzle having previously been

TABLE 3. EFFECT OF NEMBUTAL ANESTHESIA ON THE BASAL METABOLISM OF A EWE[1]

Date	Condition of animal	Fasted hrs.	Time after injection of nembutal [2] min.	Respiratory Quotient	Ventilation rate l/min.	B.M.R. (cals/24 hrs.)[3] Normal	Anesthetised	Decrease from normal percent
				Experiment I				
June 20	Normal, quiescent	72	..	0.66	5.30	847	...	...
June 20	Normal, quiescent	74	..	0.67	5.37	880		
June 20	Anesthetised	75	60	0.66	5.03	...	787	8.8
						Av. 863		
				Experiment II				
July 3	Normal, quiescent	72	..	0.66	5.04	778	...	...
July 3	Anesthetised	77	60	0.63	5.08	...	716	...
July 3	Anesthetised	78	90	0.68	5.05	...	692	9.5
							Av. 704	

[1] The body weight was about 66 lbs. during these experiments.
[2] Nembutal (1 gr./5 lbs.) was injected intravenously. The effect lasted for about three hours.
[3] Computed for an R. Q. of 0.707.

shaved and coated with vaseline. The results of three tests in the unanesthetized and three in the anesthetized condition are summarized in table 3.

The difference in heat production in the anesthetized and unanesthetized condition was 8.8 percent in one series of tests, and 9.5 percent in the other. At least part of this difference, and possibly all, was due to the unavoidable nervousness of the ewe when measurements were made without anesthesia, although the animal had been trained to lie quietly during the necessary manipulations.

In view of these results, the basal metabolism of the experimental calves was determined 30 minutes after a hypnotic dose of nembutal, i.e., 1 grain per 18 lbs. body weight. About two-thirds of the dose was injected intravenously and the rest subcutaneously. In the hyperthyroid

condition (period IX), the basal metabolism was determined about the 20th day of protamone administration, when the pulse rate had reached its maximum value.

The Minimum Endogenous Nitrogen Excretion

The minimum endogenous urinary nitrogen was determined on each calf in the normal and in the hyperthyroid state. Daily collections of urine were made in a metabolism crate; fecal contamination was avoided (or minimized) by attaching a feces bag to the rear end of the animal with appropriate harness. During the tests, the calves subsisted upon the "low-N" and "N-free" rations described in table 2 for a sufficient time so that either the nitrogen content of the day's urine per Kg. of body weight had attained a fairly constant and low value, or the percentage of the total urinary nitrogen in the form of creatinine had reached a practically constant and high value. Since the appetite of the animals declined progressively during subsistence on the "N-free" ration, the calorie intake of the animals was maintained at an adequate level by hand-feeding that portion of the day's allotment that was not consumed voluntarily (Kehar *et al.*, 1943).

Heart Rate, Respiration Rate, Rectal Temperature and Body Weight

Heart rate, respiration rate and rectal temperature of the calves were recorded every morning, beginning one week before protamone administration was started, before food and water were offered. The average of two determinations was recorded, and aberrant values were checked. The heart rate was taken by stethoscope, and the respiration rate, taken immediately after the heart rate, was determined by counting the number of flank movements per minute, care being taken not to confuse respiratory movements with rumen contractions.

The rectal temperature was taken with a clinical thermometer, with the bulb inserted about 2.5 inches inside the rectum and in close contact with the rectal wall for at least one minute. The calves were weighed before the morning feeding, almost daily.

Methods of Analysis

Urine collections were analyzed for total nitrogen by the macro-Kjeldahl method, using mercury and potassium sulfate in the digestion mixture. Preformed creatinine was determined by the method described by Hawk, Oser and Summerson (1947), total creatinine by a modification of the Folin method (Mitchell and Carman, 1926), and creatine by difference.

The analysis of gas samples collected in the course of the basal

metabolism determinations was made with the Carpenter modification of the Haldane gas analyzer (Carpenter, Lee and Finnerty, 1930), after securing, in each case, a satisfactory analysis of out-door air.

Experimental Results

Body Weight Changes

During the hyperthyroid periods, the changes in body weight of calf 965 were inconsiderable except during period XII when the "N-free" ration was fed (see table 1). In the case of calf 60, a fairly continuous loss in body weight occurred as the result of protamone feeding, amounting to 0.6 to 3.0 lbs. per day, in spite of increased feed allowance. With this calf, body weight was maintained in period XII.

Changes in Pulse Rate, Respiration Rate and Rectal Temperature

Changes occur in pulse rate, respiration rate and rectal temperature during protamone feeding. The development of hyperthyroidism was associated with elevations of all of these indices of metabolic stimulation, the maximum elevations occurring in 15 days with the pulse rate, but not for 25 to 30 days for respiration rate and rectal temperature. It is interesting to note that during subsistence on the low-N ration there was a marked tendency for the rectal temperature and the respiration rate to decline, although protamone administration continued. During this period of low-protein feeding the pulse rate declined for calf 965, but not for calf 60.

Changes in Basal Metabolism

The changes in the basal metabolism of the calves, expressed in calories per m^2 body surface per day, from the normal to the hyperthyroid condition, are summarized in table 4. Metabolism determinations were made from 72 to 96 hours after feeding. Since the respiratory quotient did not decrease consistently after 72 hours of fast, the post-absorptive condition was probably reached, under the feeding practices used in these tests, in 72 hours from the last meal. The decreases in heat production observed in later hours, particularly when the calves were in the hyperthyroid condition, may be considered as specific inanition effects. Comparing the values secured after 72 hours of fast, the basal metabolism of calf 60 was increased 35 percent by the degree of hyperthyroidism imposed, and that of calf 965, 31 percent. These increases exceed that expected from Blaxter's equation above, i.e., 25 percent.

The basal metabolism of the two Holstein bull calves in the normal condition was 1285 cals. per m^2 per day for no. 60, and 1328 cals. for no. 965, in the lying position and anesthetized with nembutal. The basal metabolic rates of animals of these weights, 141 and 110 Kgm., as predicted by the equation of Brody *et al.* (1932) for Holstein bull calves, lying in a natural position after 2 to 4 days of fast, are 1713 and 1649 cals. on the same basis. The equation used in this prediction is the one of two given yielding the lower values. Benedict and Ritzman

TABLE 4. BASAL METABOLISM OF CALVES IN NORMAL AND HYPERTHYROID PERIODS

		Calf No. 60				Calf No. 965	
Period	Fasted hrs.	Basal heat [1] cals/m^2/day	Respiratory Quotient	Period	Fasted hrs.	Basal heat cals/m^2/day	Respiratory Quotient
		Normal				Normal	
II	72	1249	0.74	II	72	1328	0.74
VII	72	1320	0.76	II	76	1298	0.71
VII	90	1102	0.75	VII	90	1291	0.78
		Hyperthyroid				Hyperthyroid	
IX	72	1739	0.80	IX	72	1738 [2]	0.65
IX	96	1571	0.82	IX	96	1555	0.70

[1] Surface area was computed by using the formula of Brody and Elting (1926): $A_{m^2}=0.15\times W^{0.56}_{kgm.}$

[2] Assuming the R. Q. to be 0.707.

reported much the same values for steers much heavier in weight, 250 to 600 Kgms., and in the standing position. On the basis of estimates of the energy cost of standing, amounting to 20 to 30 percent of the heat production of the standing animal, these investigators place the probable basal metabolism of beef steers at about 1300 cals. per m^2 per day. Forbes *et al.* (1931) found the basal metabolism of two steers, weighing 300 to 360 Kgms., to be 1217 and 1509 cals. per m^2 per standard day of 12 hours standing, or in the lying position, 1101 and 1333 cals, per m^2 per day. With the exception of Brody's predicted values, the results of the other three laboratories are in reasonably good agreement in indicating an average basal metabolism of cattle in the lying position of approximately 1300 cals. per m^2 per day, with no clear indication of an age effect within the age range studied.

Changes in the Minimum Endogenous Excretion of Nitrogen

The minimum excretions of endogenous urinary nitrogen of the calves in the normal and in the hyperthyroid states are presented in table 5. The daily excretions for the last 4 or 5 days of the nitrogen-free period,

TABLE 5. THE MINIMUM ENDOGENOUS URINARY NITROGEN OF THE CALVES IN THE NORMAL AND IN THE HYPERTHYROID CONDITION, EXPRESSED ON THE DAILY BASIS

Duration of feeding "N-free" diet[1] days	Total urinary nitrogen gm.	Creatinine gm.	Creatine gm.	Creatinine N in pct. of total urinary N	Duration of feeding "N-free" diet[1] days	Total urinary nitrogen gm.	Creatinine gm.	Creatine gm.	Creatinine N in pct. of total urinary N
		Calf 60					Calf 965		
		Normal Condition					Normal Condition		
7	8.38	4.26	....	18.9	7	5.81	3.11	....	19.9
8	6.49	3.48	0.09	19.9	8	5.61	3.16	0.11	20.9
9	9.67	5.04	....	19.4	9	5.84	3.33	0.22	21.2
10	7.80	4.29	0.16	20.4	10	5.53	3.23	0.06	21.7
11	7.11	4.02	0.31	21.0	11	6.49	3.38	0.13	19.4
Averages	7.89	4.22	0.19	19.9	Averages	5.86	3.24	0.13	20.6
		Hyperthyroid Condition					Hyperthyroid Condition		
2	7.45	3.00	1.90	15.0	3	7.43	2.67	0.15	13.4
3	8.66	3.14	2.24	13.5	4	7.13	2.81	....	14.6
4	8.19[2]	3.13	....	14.2[2]	5	6.53	2.57	0.61	14.6
5	7.75[2]	2.96	....	14.2[2]	6	5.96	2.17	0.97	13.5
Averages	8.01	3.06	....	14.2	7	6.10	2.73	0.91	16.6
					Averages	6.63	2.59	....	14.5

1 Following "low-N" feeding of 6 days in the normal periods and 20 days in the hyperthyroid periods.
2 These urines were contaminated with feces, as the calf had diarrhea. The total urinary N was computed on the assumption that the creatinine N constituted 14.2 pct. of the total, this being the average for days 2 and 3.

on which the averages are based, are much more consistent for calf 965 than for calf 60; the creatinine excretion was fairly constant for both calves. In the hyperthyroid condition the calves excreted more endogenous nitrogen at lighter body weights, but less creatinine, than in the normal condition. When computed to the square meter of body surface, the values for total endogenous nitrogen for calf 60 were 3814 mg., for the hyperthyroid period, as compared with 3288 mg. for the normal period, a 16 percent increase; for calf 965, the values were 3348 vs. 2804 mg., an increase of 19 percent.

The creatinine nitrogen per m^2 body surface decreased on the average from normal to hyperthyroid period, the values being 654 and 542 mg. for calf 60, respectively, and 576 and 486 mg., for calf 965. The marked increase in creatine excretion of hyperthyroidism indicates a considerable stimulation of the endogenous nitrogen catabolism by protamone.

Not many reports have been found in the literature of determinations of the minimum endogenous urinary nitrogen of cattle carried out under conditions of very low-nitrogen feeding. The earliest one is a report by Hart, Humphrey and Morrison (1912) describing such an experiment on two Holstein heifers weighing 158 and 172 Kgm. Computing the body surface of the calves by the formula of Brody and Elting (1926), the urinary nitrogen excretions on a low-nitrogen diet were, respectively, 2027 and 1775 mg. per m^2 body surface per day. Swanson and Herman (1943) have reported similar data on a number of pure-bred Holstein-Friesian calves ranging in body weight from 283 to 422 Kgms. The average of 15 satisfactory determinations on ten calves of the minimum endogenous urinary nitrogen excretion is 2087 mg. per m^2 per day, with individual values ranging from 1672 to 2424 mg. The results of Kehar, Mukherjee and Sen (1943) on 6 adult Indian bullocks averaged 1686 mg. endogenous nitrogen per m^2 per day. Their low values in comparison with others cited are explained as being probably associated with a tropical climate, which, in many investigations on Indian men and women has been shown to be associated with basal metabolic rates 6 to 16 percent lower than American or European.

The higher values for endogenous urinary nitrogen observed in the normal periods of this experiment, i.e., 3288 and 2804 mg. per m^2 per day for calves 60 and 965, were associated with low percentages of urinary creatinine nitrogen on the total urinary nitrogen, i.e., 19.9 and 20.6. In the article of Hart *et al.* (1912), no creatinine values are given, but in that of Swanson and Hedman (1943) the percentage of creatinine nitrogen was 29 to 30, and in the Indian experiments it ranged from 30 to 40.

It does not seem profitable in the present state of knowledge concerning the factors with reference to the animal or its environment that modify the endogenous metabolism of nitrogen, to discuss these discrepancies among the results of different experiments on cattle. We need to know more concerning the effects of age, sex, climate and previous nutrition to warrant such a discussion.

Effect of Hyperthyroidism on the Ratio of Endogenous Urinary Nitrogen to Basal Heat

The ratios of endogenous urinary nitrogen to basal heat for both calves and both experimental periods are given in table 6, together with

TABLE 6. COMPARISON OF AVERAGE RESULTS ON CALVES IN NORMAL AND HYPERTHYROID CONDITIONS

Calf No.	Average body weight Kgm.	Body surface[1] m^2	Endogenous urinary N		Creatinine		Basal metabolism per day cals/m^2	Ratio of endogenous N to basal heat mg/cal.
			Total per day gm.	Per m^2 body surface mg.	Total per day gm.	As pct. of total urine N		
				Normal Condition				
60	141	2.40	7.89	3288	4.22	19.9	1284	2.56
965	110	2.09	5.86	2804	3.24	20.6	1313	2.14
				Hyperthyroid Condition				
60	111	2.10	8.01	3814	3.06	14.2	1739	2.19
965	100	1.98	6.63	3348	2.59	14.5	1738	1.93

[1] Estimated by the equation of Brody and Elting (1926).

the basic data for ready comparison. Since the endogenous nitrogen per m^2 of body surface was elevated to a less extent by protamone feeding than was the basal metabolism, the ratio of endogenous nitrogen to basal heat decreased for both calves as the result of hyperthyroidism, though the decrease was not great for calf 965 for which greater success was realized in covering calorie requirements in the hyperthyroid condition (see table 1). In this respect the experiment on calves differed from that previously carried out on rats (Mukherjee and Mitchell, 1949). For adult rats hyperthyroidism did not disturb this ratio.

Practical Considerations

According to Blaxter *et al.* (1949), a British Friesian cow when 20 weeks in milk and producing 25 lbs. of milk daily, has been observed

to give a peak increase in daily milk production of 5.2 lbs. in response to 20 gm. iodinated casein daily. A body weight of 545 Kgm. (1200 lbs.) would correspond to a 20 gm. dose of iodinated casein according to the Blaxter equation cited above, and used in computing dosages for the experimental calves. On the basis of the results of this experiment, one may assume that such a cow would respond to 20 gm. of iodinated protein with about a 30 percent increase in her basal metabolism and about an 18 percent increase in her endogenous nitrogen loss. Without giving the intermediate calculations,[2] it may be shown that the cost of the extra 5.2 lbs. of milk, assuming 3.5 percent of milk fat, would amount to 0.65 lb. TDN and 0.065 lb. digestible crude protein per lb. These values are 2.36 and 1.53 times, respectively, the normal feed cost of a lb. of 3.5 percent milk.

Summary and Conclusion

The extra energy and protein (nitrogen) expended in basal activities of the bovine induced by the feeding of iodinated casein have been studied by appropriate experiments on two Holstein bull calves. The basal metabolism of each calf, and the minimum endogenous urinary excretion of nitrogen, were studied when the calves were in the normal unstimulated condition, and also when they had come into equilibrium, as judged by pulse rate, respiration rate and rectal temperature, with a selected dose of iodinated casein (protamone).

Under the conditions imposed, the basal metabolic rate of the calves was increased in hyperthyroidism by 31 and 35 percent, and the minimum endogenous urinary nitrogen by 19 and 16 percent, per m^2 of body surface. The ratio of endogenous nitrogen to basal calories was lowered in hyperthyroidism from 2.56 mg. to 2.19 mg. with one calf, in which body weight was lost rapidly during protamone feeding, and from 2.14 to 1.93 mg. with the other.

Thus, in experimental hyperthyroidism, both the basal expenditure of energy and the endogenous catabolism of nitrogen are elevated.

An illustration is given of the practical significance of these results, indicating that the TDN cost of the extra milk produced in response to a practical dosage of iodinated casein may be increased by more than 100 percent over the normal cost, while the cost in terms of digestible crude protein may be increased over normal by about 50 percent.

[2] These intermediate calculations may be obtained by writing to the junior author at Urbana, Illinois.

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THE VALUE OF WOOD MOLASSES AS A FEED FOR DAIRY CATTLE [1]

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FOR some time by-products of various industries have been used as feeds for livestock. Recently, wood molasses, a by-product of the lumber industry, has been developed. According to Harris (1947), one ton of wood waste will yield from 86 to 200 gallons of molasses containing approximately 50% sugar, depending upon the species and type of wood used. The relative hexose and pentose content of the molasses also varies, depending upon the wood used. For example, molasses made from Douglas fir contains about 80% hexoses and 20% pentoses; however, with some of the hard woods the content is 60% hexoses and 40% pentoses.

At present, only a limited amount of information is available on the value of wood molasses as a feed. McGinnis *et al.* (1948) found that wood molasses may be substituted satisfactorily for cereal grains in the diet of the chick when additional protein is added in the form of soybean oil meal. Levels of wood molasses up to 20% had no laxative effect, but the higher levels depressed chick growth slightly, although not significantly.

Jones (1949), in trials with 12 heifers, reported that the dry matter of wood sugar molasses was as well utilized as the dry matter of cane molasses. The same author studied alfalfa silage preserved with cane molasses, as compared with alfalfa silage preserved with wood molasses, in a reversal type trial with two groups of 11 cows each and lasting a total of five weeks. Cows refused 22.5% of the cane molasses silage offered and 14.5% of the wood sugar molasses silage. There was no appreciable difference between the two groups of cows in average daily milk production.

Colovos *et al.* (1949) have reported recently the value of wood molasses as determined by balance experiment using 6 dairy heifers. These authors found that both wood and cane molasses contained essentially the same amount of metabolizable energy per gram of dry

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[2] Department of Dairy Husbandry. The authors are grateful to the United States Forest Products Laboratory, Madison, Wisconsin, for furnishing the wood waste molasses used in this experiment.

matter. They conclude that wood molasses is comparable to cane molasses as a feed for dairy cattle.

Two experiments are reported in this paper. Experiment I was conducted to determine whether or not wood molasses had any nutritive value for growing dairy heifers. At the time of initiation of this trial this fact had not as yet been established. Experiment II was designed to study the use of wood molasses as a silage preservative.

Experimental Procedure and Results

Experiment I

For experiment I, 10 heifer calves (8 Holsteins and 2 Jerseys) ranging in age from 3 to 10 months were divided into 2 groups. Animals were paired on the basis of breed, weight, and age.

Group I was designated as the molasses group and group II as the non-molasses group. The first 8 weeks of the trials were designated as phase I and the next 7 weeks as phase II. Both groups received 3 pounds of grain daily and enough chopped mixed hay of fair quality so that they were being fed at a level 5% below Morrison's (1942) minimum standards for growing dairy cattle. In addition, group I received 2 pounds of wood molasses daily during phase I and 4 pounds of wood molasses daily during phase II.

The purpose of employing the procedure of feeding 5% below Morrison's minimum standards was to make more easily discernible any nutritive value possessed by the wood molasses. As the experiment was set up, any differences in weight gains in favor of group I should have been directly attributable to the molasses which they received.

As the trial progressed it became obvious that the calves receiving wood molasses were gaining at a somewhat more rapid rate than those receiving no molasses. As a consequence, the level of wood molasses was increased to four pounds daily to determine whether weight gains at a higher level of molasses would be more rapid, whether higher levels would influence acceptance of molasses by the calves, and whether any undesirable physiological effects would result from the higher level of molasses supplementation.

The grain mixture fed to all calves contained approximately 16% crude protein. Calves were individually fed and the T.D.N. requirement for each calf was recalculated weekly on the basis of its weight.

Weights and height at withers were recorded on 3 consecutive days at the start and conclusion of the experiment. During the course of the experiment, calves were weighed and measured weekly. When taking

height at withers, 3 measurements were taken and the average of the 3 recorded. Records of feed consumption were kept for each calf. The results of experiment I are summarized in table 1. During phase I, 560 lbs. of wood molasses caused an advantage in weight of 79 lbs. in favor of the calves in group I. During the second phase, 980 lbs. of wood molasses produced a weight advantage of 76 lbs.

A statistical analysis of the data made according to the methods outlined in Snedecor (1946) showed that the advantage in weight gains for the wood-molasses-fed calves, during both the first and second phases, was significant at the 1% level of probability. The

TABLE 1. COMPARATIVE AVERAGE DAILY NUTRIENT INTAKES AND WEIGHT GAINS OF TWO GROUPS OF DAIRY HEIFERS RECEIVING, RESPECTIVELY, RATIONS WITH AND WITHOUT MOLASSES

Group	Phase I (8 weeks)			Phase II (7 weeks)		
	T.D.N. from hay & grain	Molasses fed	Weight gains	T.D.N. from hay & grain	Molasses fed	Weight gains
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
I (5 calves)	5.77	2.0	1.22	6.32	4.0	1.37
II (5 calves)	5.80	—	0.94	6.20	—	1.06

differences between the two groups in increase in height at withers were not significant.

The wood molasses used in this trial did not appear to be very palatable to the calves. As the trial progressed, the calves seemed to become more accustomed to its taste. However, only one animal exhibited a liking for wood molasses such as is normally shown for cane or beet molasses.

The weight of the calves in the non-molasses group averaged 93% of the Ragsdale (1934) standard at the beginning of the experiment. At the end of phase I average weight had declined to 88.0%, and at the end of phase II to 87.4% of the Ragsdale standard. The body weight of calves which received wood molasses averaged 92.9% of the Ragsdale standard at the beginning of the experiment, 91.4% at the end of 8 weeks, and 92.9% at the close of the 15 week experimental period.

Experiment II

For the purpose of this experiment, 16 cows were divided into 2 groups of 8 each as evenly as possible in regard to breed, stage of

lactation, stage of gestation, daily production of 4% fat-corrected milk, and body weight. Group I was designated as the wood molasses silage group and group II as the non-molasses silage (control) group. Unfortunately, one cow originally selected for the non-molasses silage group developed a kidney infection about midway in the experiment, and data obtained from this cow were discarded.

The material ensiled was a mixture consisting of 40% of alfalfa and 60% of a mixture of smooth brome and orchard grass. Both the molasses and non-molasses silages were prepared from the same field at the same time and were ensiled in upright silos. Wood molasses was diluted with water for ease in handling and was added to the one silo at the rate of 60 lbs. of molasses per ton of green material. The non-molasses silage was ensiled by the usual wilting procedure with no preservative.

At the time the trial was initiated, both the molasses and non-molasses silage had been stored in their respective silos for 10 months. Cows were fed silage at the rate of 3 lbs. per hundred lbs. of body weight, and grain at the rate of 1 lb. to each 3 lbs. of 4% fat-corrected milk. Amounts of silage and grain fed were adjusted weekly according to milk production and body weight changes. Chopped alfalfa hay was fed *ad libitum*.

Hay, silage and grain weighbacks were made routinely, and were analyzed. Nutrient consumption was ascertained by subtracting nutrients in the weighbacks from nutrients fed. Milk weights were recorded routinely, and composites were made and analyzed at the end of each experimental week for percentage of butterfat. Cows were weighed on 3 consecutive days at the beginning and end of the experiment and weekly on 2 consecutive days during the course of the experiment.

The trial was originally set up as a single reversal trial lasting 12 weeks, with 6-week experimental periods and a 1-week transition period. However, because of limited silo space and silo filling operations, it was necessary to feed corn silage instead of the non-molasses grass silage during the reversal phase of the experiment. Only data comparing the wood molasses preserved grass silage to the non-molasses grass silage are presented herein. Certain of the data collected during the reversal phase of the experiment have some value in evaluating such things as palatability of the wood molasses silage, and that information will be referred to subsequently.

The gross chemical analyses of the two silages fed in this experiment are presented in table 2. The wood molasses silage was in a somewhat better state of preservation and had a slightly better aroma

TABLE 2. GROSS CHEMICAL ANALYSES AND T. D. N. ON AN AS-FED BASIS OF SILAGES MADE WITH AND WITHOUT THE ADDITION OF WOOD MOLASSES AS A PRESERVATIVE

Silage	Dry Matter	Crude Protein	Ether Extract	Crude Fiber	Ash	N.F.E.	T.D.N.
	%	%	%	%	%	%	%
Non-molasses legume-grass	27.9	2.3	0.8	11.0	2.5	11.3	15.5
Molasses legume-grass	23.1	1.6	0.9	10.5	2.7	7.4	12.6

than the material ensiled with no preservative. The wood molasses silage was slightly darker in color. Both silages were quite palatable to the cows.

The group receiving wood molasses silage refused 5.8% of the silage which they were fed, while the non-molasses silage group had 6.6% refusals during the initial 6-week period. The group initially fed non-molasses silage had only 2.9% refusals during the 4-week period after they were switched to wood molasses silage. As explained previously, the group which had received molasses silage during the first

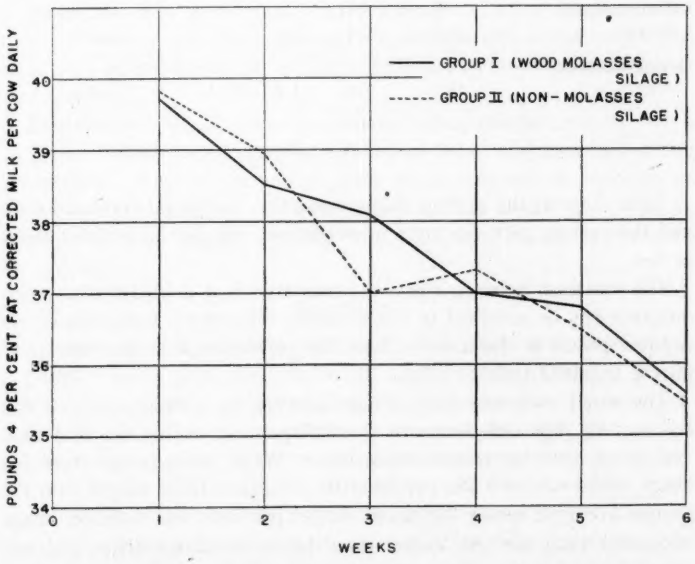

Figure 1. Average lactation decline for 6-week experimental period.

6 weeks of the experiment was switched to corn silage for the next phase of the experiment, so no valid comparison can be made as regards relative palatability of the 2 silages for this group of cows. If any advantage existed in palatability between the 2 silages, that advantage was on the side of the wood molasses preserved silage.

The average daily production per cow of 4% fat-corrected milk during each experimental week for each of the 2 groups of cows is shown graphically in figure 1. As can be seen from figure 1, the two groups of cows started at essentially the same level of 4% fat-corrected milk, and the production of the 2 groups was closely parallel during the course of the experiment.

TABLE 3. AVERAGE DAILY NUTRIENT INTAKE AND PERFORMANCE OF DAIRY COWS FED RATIONS CONTAINING DIFFERENT TYPES OF SILAGES

Dietary Group	T.D.N. Consumed			Milk production and wt. changes	
	Grain & hay	Silage	Total	4.0% F.C.M. daily	Changes in body weight during 6-week period
	lbs.	lbs.	lbs.	lbs.	lbs.
I Wood-molasses silage[a]	15.6	4.4	20.0	37.7	+8.3
II Non-molasses silage[b]	16.2	5.6	21.8	37.4	—8.5

[a] 8 cows.
[b] 7 cows.

Table 3 shows the average daily production of 4% fat-corrected milk and the average gain (or loss) in weight per cow for the entire 6-week period.

The variation between cows was such that it is doubtful if any significance can be attached to the observed differences. Furthermore, no reversal period is available to check the performance of the same cows on the opposite type of silage.

The wood molasses silage group received an average of 1.21 lbs. less of total digestible nutrients from silage daily during the trial than the group receiving non-molasses silage. While both groups were fed silage at the rate of 3 lbs. per hundred pounds of body weight, and the groups averaged nearly the same weight per cow, the molasses silage contained 4.8% less dry matter than the non-molasses silage, and had 2.9% less total digestible nutrients per pound on as-fed basis. This

accounts largely for the difference in total digestible nutrient consumption of the 2 groups.

Slight differences in the efficiency of total digestible nutrient utilization in favor of the wood molasses silage fed group were observed. However, errors inherent in the determination of digestion coefficients (and thus T. D. N.) were probably sufficient to render the observed differences non-significant.

Discussion

The major drawback to the wood molasses used in experiment I was its lack of palatability. As might be expected, there was considerable individual variation between calves in their liking for molasses. Two of the animals receiving molasses never seemed to relish it.

One of the barrels of molasses from which a sample was taken had a pH of 4.1. According to Harris (1949), when large amounts of bark are present in the wood waste from which molasses is made, tannin-like substances may be found in the molasses in greater than normal amounts, imparting to it a rather bitter taste. Part of the molasses in one barrel used in preliminary trials had been made from southern pine which had been solvent extracted for terpins and resins. This molasses was very unpalatable to the animals to which it was offered. One or more of the above factors, or perhaps others not mentioned here, may contribute to the lack of palatability of wood molasses which was observed.

Experimental studies reported herein further substantiate the work of Jones (1949) concerning the efficacy of wood molasses as a silage preservative. The forage ensiled with wood molasses as reported in experiment II produced a silage which had a slight advantage in palatability and keeping qualities when compared to silage produced by wilting.

Summary

To determine the nutritive value of molasses made from wood waste, a paired feeding trial was conducted using 8 Holstein and 2 Jersey heifers divided into 2 groups of 5 each. Calves were fed at a level 5% below Morrison's minimum standards for growing dairy cattle. Five of the calves received 2 lbs. of wood molasses daily during the first 8 weeks of the trial and 4 lbs. daily during the last 7 weeks. Calves receiving the wood molasses gained significantly more weight than did the non-molasses group. There was no significant difference in increase in height at withers between the 2 groups.

In order to study the use of wood molasses as a silage preservative, a grass-legume mixture was ensiled with 60 pounds per ton of wood molasses. A feeding trial was run lasting 6 weeks and using 2 groups of cows, 8 in one group and 7 in the other. One group was fed the wood molasses preserved silage and the second a silage prepared from the same field at the same time, but ensiled with no preservative. The criteria in this trial were production of 4% fat-corrected milk, maintenance of body weight, efficiency in use of total digestible nutrients, and palatability and keeping quality of the silage. The silages were nearly equal in most respects, but the silage preserved with wood molasses seemed to be slightly more palatable and better preserved than that to which no preservative had been added.

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COMPARATIVE STUDY OF CONVENTIONAL PROTEIN ALLOWANCES AND THEORETICAL REQUIREMENTS OF GROWING HOLSTEIN HEIFERS

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THE protein allowance of current feeding standards for growing dairy heifers is based upon meager experimental data. Loosli *et al.*, (1945) and Lofgreen (1948) have reviewed in some detail the earlier research which formed the basis for the protein allowances of the present feeding standards used in this country. These standards are based largely on the results of feeding trials. Some years ago Mitchell (1929) proposed a different approach for estimating requirements. He calculated the theoretical minimum protein requirement for growing heifers by adding the amounts required for maintenance and for growth. The amount required for maintenance was estimated from endogenous urinary nitrogen losses and the amount for growth was calculated from the nitrogen content of the gains of growing beef steers. Blaxter and Price (1946) recently have tested calculated values closely approximating Mitchell's original estimates and have shown them to be inadequate for normal growth. A few years ago Mitchell recalculated the estimates by the factorial method using a maintenance requirement based upon the work of Smuts (1935) and an expected daily gain based upon the data of Espe *et al.* (1932). These calculations were issued in mimeographed form and published by Maynard (1947). Estimates made by the use of these newer values allowed 1.01 pounds of digestible protein for a 150-pound calf with a decrease to 0.61 pound for a 1000 pound animal. By way of comparison the 1936 Morrison feeding standard allows 0.46 and 1.24 pounds of digestible crude protein for these two weights, respectively. Because these calculated minimum requirements differed so markedly from the allowances of conventional feeding standards and because they had never been tested in actual feeding trials, it appeared worth while to feed animals according to these calculations and compare their response with those fed the allowances of the Morrison standard. Actually the values calculated by the factorial method are not allowances to be used as a feeding

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standard but are theoretical minimum requirements. A minimum requirement probably should never be fed in practice but determining the minimum needs often helps to put feeding standards on a more scientific basis.

After this study was begun, Blaxter and Price (1946) and Blaxter and Mitchell (1947) pointed out that the original factorial method as used by Mitchell failed to take into consideration the amount of protein necessary to replace the metabolic protein lost in the feces. Blaxter and Mitchell (1948) therefore recalculated the theoretical minimum requirements taking into consideration this additional factor. These latest values allowed approximately 0.90 and 0.78 pound of digestible protein for a 200 and a 1000 pound heifer, respectively. These values still differed greatly from the Morrison standard.

In an attempt to bring together these differing views experiments were carried out (Lofgreen, 1948) to give a direct comparison of the allowances of the Morrison standard (Morrison, 1936) and Mitchell's calculated minimum requirements (Maynard, 1947), and simultaneously to obtain experimental data on the protein requirements of growing Holstein heifers.

Experimental Procedures and Results

Fourteen purebred and four grade Holstein heifers were placed at random on either the protein allowance of the Morrison standard or on the factorially calculated requirements of Mitchell at 150 pounds of body weight and grown to 350 pounds. The amounts of hay and concentrate to be allowed at the various weights were determined in advance from unpublished consumption data (Norton, 1945). The percentage crude protein was determined in the hays to be used and the digestible protein estimated by the use of average digestion coefficients. It was then possible to calculate the amounts of digestible protein necessary in the concentrate mixtures. The actual consumption of digestible protein and total digestible nutrients was determined from digestion coefficients obtained on a steer balance study using the same feeds as were fed the heifers in this experiment.

Weekly weights were taken and measurements of height at withers and circumference at heart girth were made every two weeks. Feed adjustments were made at intervals of ten pounds in body weight. Weighings were made each week on the same day and at the same time of day in relation to feeding. On weigh days water was withheld until after the weighing was completed. It has been shown (Baker, *et al.*,

1947) that under uniform conditions one-day weights are as satisfactory as the average of three days' weights.

The first ten calves placed on experiment were fed the amounts of total digestible nutrients recommended in the 1936 Morrison standard. The gain in weight was below normal for all calves regardless of protein level. The energy allowance was therefore increased for the remaining calves. As an average, the total digestible nutrient intake was approximately 105 percent of the Morrison standard.

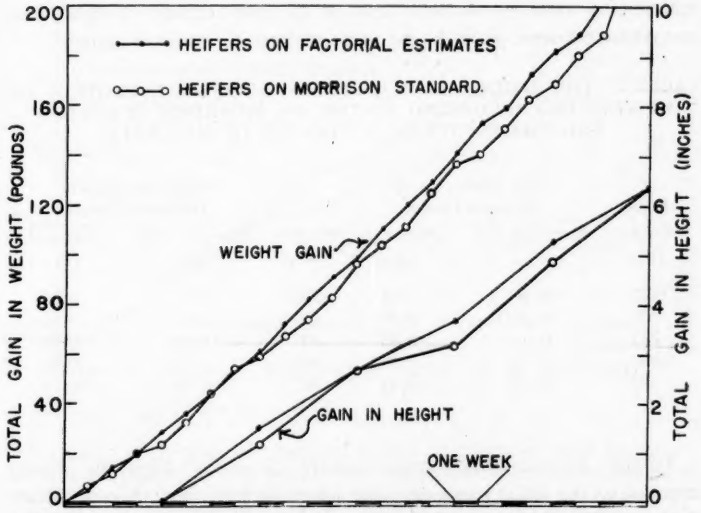

Figure 1. The cumulative gains in body weight and height at withers of heifers in Experiment I (9 heifers per group).

An analysis of the gains presented in figure 1 reveals that the addition of more protein than is called for in the Morrison standard gave no significant increase in growth response. The factorial groups held a slight advantage in weight but the differences were not significant. The lowest protein intakes which produced maximum gains at the weights of 150, 200, 250, 300 and 350 pounds were 0.36, 0.47, 0.62, 0.72 and 0.79 pound of digestible protein daily. At 150, 200 and 250 pounds these intakes are below the recommendations of the Morrison standard. At 250, 300 and 350 pounds these levels were the lowest fed and it was therefore merely shown that they were adequate.

Another group of 13 purebred Holstein heifers was used in the phase

of the study, which began at an average body weight of 550 pounds, the point at which the allowances of the Morrison standard and Mitchell's calculated minimum requirements do not differ markedly. By random selection seven of the heifers, averaging 535 pounds, were placed upon the level called for in the Morrison standard and the remaining six, averaging 575 pounds, were started on Mitchell's theoretical minimum requirements. The protein allowances for both groups are presented in table 1. Both groups were fed the total digestible nutrient allowances of the Morrison standard. The animals were fed individually and records were kept of all feed refusals. Weights and measurements were made in the same manner as outlined above.

TABLE 1. THE DAILY ALLOWANCES OF DIGESTIBLE PROTEIN OF HEIFERS FED ACCORDING TO THE 1936 MORRISON STANDARD AND THE FACTORIAL ESTIMATES OF MITCHELL

Body Weight	Daily Allowance of Digestible Protein		Body Weight	Daily Allowance of Digestible Protein	
	Morrison Std.	Factorial		Morrison Std.	Factorial
(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
150	0.46	1.01	500	0.92	0.86
200	0.57	0.97	600	1.00	0.81
250	0.66	0.95	700	1.06	0.76
300	0.72	0.94	800	1.13	0.71
400	0.85	0.91	900	1.19	0.66
			1000	1.24	0.61

Timothy hay containing approximately 2.0 percent digestible protein was fed to the heifers receiving the lower protein level. A concentrate mixture containing the proper amount of protein was given at the rate of approximately 4 pounds per day. The group being fed the protein allowances of the Morrison standard was fed timothy hay containing 4.7 percent digestible protein and a concentrate mixture containing the proper percentage of protein to achieve the desired protein level. All concentrate mixtures were made by varying the proportions of corn, wheat bran, ground oats, soybean meal, linseed meal and, in a few cases, dried beet pulp and dextrose. The digestible protein content of the mixtures was estimated by applying average digestion coefficients to the determined crude protein content of the individual ingredients. Throughout the study a carotene supplement was given to the heifers receiving the poor quality hay and all animals were allowed free access to a mineral mixture consisting of 2 parts salt, 1 part limestone and 1 part dicalcium phosphate.

From an inspection of figure 2 it becomes clear that heifers fed digestible protein of the factorial estimates gained at a normal rate until they reached 730 pounds in body weight. Thereafter the growth rate remained subnormal, showing that 0.76 pound of digestible protein daily was inadequate.

After 44 weeks, seven heifers which had not reached 1000 pounds were changed to a ration furnishing approximately 0.90 pound of digestible protein daily since on the lower protein allowance growth

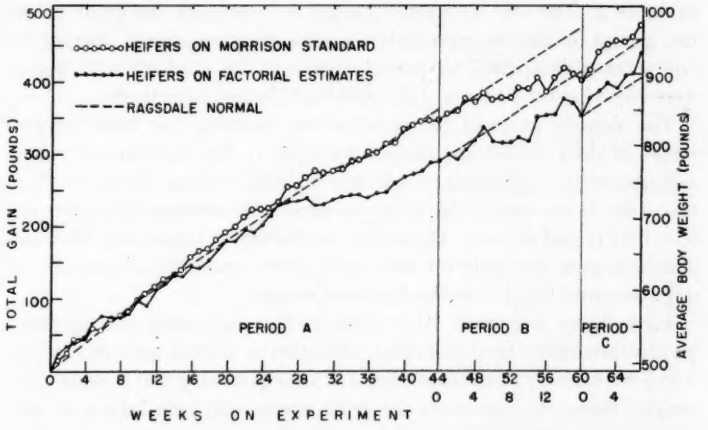

Figure 2. The cumulative gains of heifers as compared with Ragsdale's normal. During period A 7 heifers were fed digestible protein according to the Morrison standard and 6, the Factorial estimates (table 1). In period B both groups received 0.90 pound of digestible protein and in period C both received 1.00 pound daily.

was subnormal. Because of the small numbers available it was not possible to leave control animals in each group. Four heifers which previously had been fed according to the Morrison standard and three which had been receiving the lower intake of the factorial system were fed this new level for a period of 16 weeks. The average initial weight of the group of seven heifers was 813 pounds.

At the end of this period all heifers were again changed to a ration calculated to furnish approximately 1.00 pound of digestible protein per day. This level was fed for a period of seven weeks. The average weight at the beginning of the period was 867 pounds.

The individual growth data revealed a marked difference in the

response of pregnant and open heifers to the lower protein intakes. Unbred heifers ceased gaining weight at about 730 pounds body weight whereas pregnant heifers continued to gain at a normal rate on the same protein intakes until they weighed 850 pounds or more. Whether pregnant heifers are more efficient than unbred heifers in utilizing protein or if their weight gains were due to increases in fetal tissues and fluids occurring at the expense of the heifers' bodies is not clear.

Because of the difference in the response of the heifers to the lower protein intake the gains of the open heifers only have been presented in figure 2. The first 44 weeks (period A) represent the gains of the two groups on the two protein levels while the next sixteen (period B) and seven week (period C) periods represent the gains when all heifers were receiving the 0.90 and 1.00 pound allowance respectively.

The average gains of the open heifers receiving the lower protein intake of the factorial calculations are equal to the Ragsdale normal to approximately 730 pounds, at which point they decrease abruptly. From this point to the end of the 44 weeks period the average daily gain was only 0.47 pound per day. The heifers on the higher intake, the Morrison standard, grew normally for the entire period, gaining 1.15 pounds per day compared to 1.18 for the Ragsdale normal.

From figure 2 (period B) it appears that increasing the digestible protein allowance to 0.90 pound permitted a normal growth rate for 4 to 5 weeks or to an average weight of approximately 850 pounds. This weight, therefore, represents the point above which an intake of 0.90 pound of digestible protein daily appeared to be inadequate to support normal growth. After 850 pounds all heifers grew subnormally on this intake.

On 1.00 pound of digestible protein per head daily (period C) the gains of all heifers are clearly equal to or above the Ragsdale normal. It is recognized that a seven weeks period is too short a time upon which to base definite conclusions, but it is long enough to indicate a definite improvement in the rate of gain.

Discussion

The values, which these experiments indicate closely approach the minimum requirement, are summarized in table 2 and compared with the Morrison feeding standard. No data are available for the weight of 400 pounds.

Since 0.75 pound of digestible protein seemed to be inadequate for heifers at approximately 730 pounds and since they grew at a normal

rate to 850 pounds on 0.90 pound it seems logical to assume that 0.90 pound of digestible protein daily is close to the minimum requirement from 700 to 800 pounds for the heifers and the feeds used in this experiment. It also seems logical that 0.80 pound would be adequate from 500 to 700 pounds. Because 0.90 pound is inadequate for an 850 pound heifer and normal or above normal gains can be obtained from 850 to 950 pounds on 1.00 pound, it seems likely that under the conditions of this study 1.00 pound of digestible protein daily approaches the minimum requirement from 900 to 1000 pounds.

TABLE 2. OBSERVED MINIMUM DAILY REQUIREMENTS OF DIGESTIBLE PROTEIN COMPARED WITH THE MORRISON STANDARD

Body Weight	1936 Morrison Standard	Observed Requirements	
		Digestible Protein	Crude Protein
(lb.)	(lb.)	(lb.)	(lb.)
150	0.46	0.36	0.58
200	0.57	0.47	0.76
250	0.66	0.62	0.98
300	0.72	0.72	1.07
400	0.85	—	—
500	0.92	0.80	1.27
600	1.00	0.80	1.32
700	1.06	0.90	1.48
800	1.13	0.90	1.50
900	1.19	1.00	1.67
1000	1.24	1.00	1.70

Even though these data are limited and cannot be taken as absolute minimum requirements, they do indicate that the allowances of the Morrison feeding standard for the Holstein heifers used were adequate and that they could probably be lowered somewhat for heifers weighing 600 pounds and above. Since this study was completed, Morrison (1948) has made such a revision in his feeding standard for growing dairy heifers.

It is interesting to compare these results with the theoretical minimum requirements calculated by Blaxter and Mitchell (1948) who include a correction for the replacement of metabolic protein losses. According to these calculations the minimum requirement of a 200 pound calf would be 0.90 pound of digestible protein daily while the experimental data presented in this paper show no advantage of feeding more than 0.46

pound. At 800 and 1000 pounds the calculated requirements are 0.82 and 0.78 pound while the experimental data indicate these levels are inadequate.

The factorial method, although it may be theoretically valid, is no nearer accurate than the values available for the individual factors involved. A number of these factors are subject to error since the experimental data are by no means complete and are often contradictory.

The maintenance factor is calculated by use of the value of 2 milligrams of endogenous nitrogen excretion per kilocalorie of basal heat (Smuts, 1935). Ashworth (1935) and Ashworth and Brody (1933) found that with rats the nitrogen excretion may decrease up to sixty days on a nitrogen-free diet. It was found that for young rats as small an amount as 0.7 milligram of nitrogen per kilocalorie of basal heat was eliminated. The average amount was about 1.4 milligrams per kilocalorie. Deuel, *et al.*, (1928) found that the human responded in much the same manner and that at the end of sixty days on a nitrogen-poor diet approximately 1.1 milligrams of nitrogen was eliminated per kilocalorie of normal basal heat. Obviously, the problem of minimum endogenous nitrogen excretion and its significance needs further investigation, especially with respect to growing farm animals. Since, however, this is being used as a basis for estimating the maintenance requirement it seems that a lower value might well be used. The value of two milligrams may be too high since it was assumed by Smuts that the animals used reached the endogenous level after a short time on a nitrogen-free or a nitrogen-poor diet.

A second factor which may lead to error is the estimation of the growth requirement for the young calves. The calculated expected daily gain for 150 and 200 pound calves is 2.08 and 2.02 pounds, respectively. Although gains of this magnitude may sometimes be obtained they are not usual. According to Ragsdale (1934) Holstein heifers weighing 150 and 200 pounds will gain an average of 1.5 and 1.7 pounds per day. The National Research Council's recommended allowances for dairy heifers (Loosli *et al.*, 1945) lists an expected gain of 1.4 and 1.6 pounds for these two weights. The reason for the over-estimation of the expected daily gain for calves of these weights is that the equation describing the growth curve does not fit the data at the early ages during the period of accelerating growth. It is probable that the same age equivalence relationship for animals following the point of inflection of the growth curve does not hold true for that period of growth before this point, a period characterized by accelerating growth. If this is true the estimates of the nitrogen stored by animals during this period would be in error.

A third source of error lies in the assumption of an average biological value for the entire growth period. Blaxter and Mitchell (1948) used a value of 60.8 in all calculations. Lofgreen, *et al.*, (1947) have pointed out that there are numerous exceptions in the literature and that the value of various proteins for ruminants may vary considerably from a mean of 60. A number of biological values as high as 80 are recorded. Briggs *et al.*, (1946) have reported values determined with calves of 76, 68, 71 and 73 for the protein in rations containing cottonseed meal, peanut meal, soybean oil meal, and a mixture of these three supplements. Unpublished data from this laboratory have shown that for calves between 200 and 300 pounds the biological value of the protein in a ration containing timothy, corn and linseed meal was 76. Savage and McCay (1942) cite instances where the young calf was able to convert 80 to 90 percent of milk protein into body tissue. Swanson and Herman (1943) have reported biological values averaging 77 for various combinations of feeds when fed to calves. Ritzman and Colovos (1943) found that when a calf was fed milk primarily, it utilized 96 percent of the digested protein. Assuming the same value for milk during the first four months, they found the protein of the calf meal and hay decreased to 79, 59 and 50 percent utilization. It seems logical that the biological value may easily approach 80 for the protein in the rations of calves on milk and a good quality, palatable calf starter. As an animal matures, consumes a higher proportion of hay to concentrate, no longer receives milk, and receives a smaller variety of proteins, the biological value undoubtedly decreases. It also seems possible that the biological value of a given protein may change depending upon the use to which it is put in the animal body. Certainly as an animal matures the use to which the absorbed protein is put changes. These data support a higher biological value for young calves than 60 and perhaps a lower one for older animals. Based upon the thesis that the biological value of various feeds for ruminants is constant Blaxter and Mitchell (1948) have suggested the term 'available protein' to replace the more common 'apparently digestible protein' in the expression of protein requirements. Expressions of available protein are, however, subject to a biological value and, as in the case of requirements expressed as apparently digestible protein, may vary depending upon the ration being fed.

Table 3 lists the requirements as calculated by the factorial method with the inclusion of various changes in some of the factors which have just been discussed and explained in the footnotes to table 3.

Figure 3 presents in graphic form a comparison of this set of calculated minimum requirements with those of Blaxter and Mitchell (1948)

TABLE 3. FACTORIAL CALCULATION OF THE PROTEIN REQUIREMENTS OF GROWING HOLSTEIN HEIFERS

Body Weight	Age	Average Daily Gain	Endogenous Nitrogen Catabolism per day (X 6.25). Maintenance	Nitrogen Stored Daily (X 6.25) Growth	Net Protein Requirement for Maintenance and Growth	Biological Value	Available Protein Requirement for Maintenance and Growth	Dry Matter Consumption	Metabolic Protein Loss	Metabolizable Protein Requirement	Apparently Digestible Crude Protein Requirement per Head per Day
lbs.	Mos.	lbs.	gms.	gms.	gms.		lbs.	lbs.	lbs.	lbs.	lbs.
					Columns						
1	2	3	4	5	6	7	8	9	10	11	12
150	2.2	1.40	20	136	156	80	0.43	3.5	0.10	0.55	0.45
200	3.0	1.60	21	151	172	75	0.50	5.1	0.14	0.69	0.55
400	6.6	1.76	40	154	194	65	0.66	9.5	0.27	1.07	0.80
600	10.6	1.52	49	118	167	60	0.61	12.9	0.36	1.21	0.85
800	15.4	1.26	56	86	142	57	0.55	16.2	0.46	1.36	0.90
1000	21.3	1.01	62	56.5	118.5	55	0.47	19.2	0.54	1.45	0.91

Columns:

1 and 2. Taken from Mitchell's values (Maynard, 1947) which he obtained from the growth data of Espe, Cannon and Hansen (1932).

3. The expected daily gain at the weights of 150 and 200 pounds were taken from the National Research Council's Recommended Nutrient Allowances for Dairy Cattle (Loosli, *et. al.*, 1945). The others are those calculated by Mitchell from the growth data referred to in 1 and 2 above.

4. Calculated on the basis of 1.5 milligrams of endogenous nitrogen excretion per kilocalorie of basal heat.

5. Taken from Mitchell's calculations of nitrogen retention of growing heifers at these weights (Maynard, 1947) with the exception that the values for 150 and 200 pounds have been reduced in proportion to the reduction of the expected daily gains from 2.08 and 2.02 to 1.40 and 1.60 for these two weight groups, respectively.

6. Sum of 4 and 5.

7. Estimated biological values based upon data discussed in text.

8. Obtained by dividing the net protein requirement by the biological value and converting to pounds.

9. The average dry matter recommendation of the Morrison feeding standard for these weights.

10. Obtained by assuming an average of 0.45 gram of metabolic nitrogen loss per 100 grams of dry matter intake (Blaxter and Mitchell, 1948).

11. These values are obtained by adding to the available protein requirement of column 8 enough protein to replace the metabolic protein loss. It is:

$$\text{Available protein} + \frac{\text{Metabolic Protein Loss}}{\text{biological value (as a decimal)}}$$ (Blaxter and Mitchell, 1948)

This represents the requirement for truly digestible protein.

12. Obtained by subtracting the metabolic protein loss from the requirement for truly digestible protein. This converts the requirement to apparently digestible protein, the conventional term for the expression of protein requirements of most farm animals.

and with the protein allowances of the 1936 Morrison feeding standard. The experimental data presented in table 2 support the changes made in the factorial calculations.

For the calculation of the minimum requirements of Holstein cattle the factorial method could lead to erroneous results and until more data are obtained on the various factors involved this method cannot be

accepted without reservation. Reliance must still be placed on the results of actual feeding trials. When good values are obtained which can be used in the factorial calculations this method will be a very useful tool in the determination of nutritional requirements.

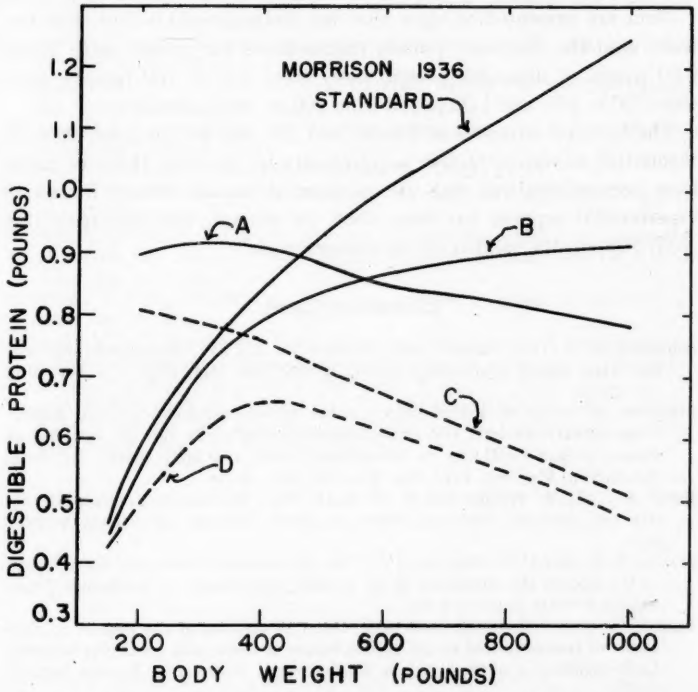

Figure 3. Comparison with the Morrison feeding standard of the Factorial estimates made in this paper and those of Blaxter and Mitchell (1948). A. Factorial estimates, Blaxter and Mitchell; B. Factorial estimates, this paper; C. Available protein requirement, Blaxter and Mitchell; and D. Available protein requirement, this paper.

Summary

Studies are reported in which it was attempted to compare the response of Holstein heifers grown on the protein allowances of the 1936 Morrison feeding standard and on the estimates of Mitchell calculated by the factorial method. The growth data indicated there was no advantage in supplying more protein to Holstein calves from 150 to

350 pounds body weight than recommended in the 1936 Morrison standard. An intake somewhat below the lower limit of this standard produced as good gains as did higher levels. For heifers weighing more than 700 pounds the digestible protein allowances of Mitchell's estimates proved grossly inadequate.

Data are presented to show that for Holstein heifers and with the feeds used the minimum protein requirements for growth were about 0.80 pound of digestible protein daily from 500 to 700 pounds, 0.90 from 700 to 800, and 1.00 pound from 800 to 1000 pounds.

The factorial estimates of Blaxter and Mitchell for the calculation of theoretical minimum protein requirements for growing Holstein cattle have been recalculated with the inclusion of various changes for which experimental support has been cited. In general, the data from this study support the validity of the changes made.

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CATTLE SHADES

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THE mean monthly temperature in Imperial Valley is above 75° for about six months of the year, exceeds 85° F. for nearly 4 months, and reaches 90° F. for two months. The high temperature is associated with low humidity until about July 15, at which time the prevailing winds shift from the west to the south and bring moist air into the area from the Gulf of Lower California.

High summer temperatures and relative humidity make cooling even for sweating animals difficult. Non-sweating temperate-zone cattle have difficulty with heat dissipation when air temperatures reach 70 to 80° F. (Regan and Richardson, 1938; Bonsma, 1940; Rhoad, 1942; Ragsdale, Brody, Thompson, and Worstell, 1948). To study the physiological responses of cattle to various types of artificial shades, investigations were started in 1946 at the Imperial Valley Field Station of the University of California.

Eleven different types of shades were tested during the summers of 1947, 1948, and 1949. Hereford and Hereford-Brahman cross-bred cattle were used in the feeding trials and for measuring the effect of the shades on the physiological reactions. Weight gains, feed consumption, respiration rates, air temperature, and amount of radiant heat entering the area under the shades were recorded at intervals.

A preliminary report on the 1947 studies discussed the results of the first four shades tested, especially with regard to the heat transfers involved (Kelly and Ittner, 1948). The types of shades, experimental procedure, and results for 1948 and 1949 will be discussed in this paper.

1948 Results

Five shades were tested in the summer of 1948. No. 1 was a galvanized-iron, open shade, 16 x 24 feet, and 10 feet high. It was used as the check. Shade No. 2 was enclosed on three sides, the open side being toward the north. The roof of aluminum sheets was laid over

[1] The authors wish to express their appreciation to Professor H. R. Guilbert, Animal Husbandry Division, and T. E. Bond, U.S.D.A., for their help in the preparation of the paper and to Emmett Layton, Assistant Herdsman.

boards; beneath it for insulation was a layer of old hay 3 feet thick, supported on woven wire. The walls were constructed of a double layer of woven wire fencing nailed to a vertical 6- x 6-inch posts and packed with hay. An evaporative air cooler ("desert cooler") with a rated capacity of 2500 cubic feet of air per minute was installed in the south end of the shade near the roof. Inside dimensions were 13 x 22 feet, with a height of 8 feet (figure 1).

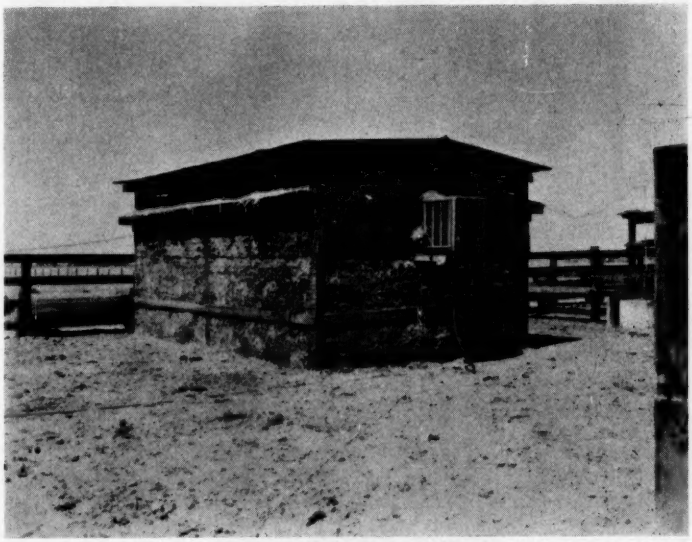

Figure 1. Three sided shade, open on north side, kept cool with evaporative (desert) cooler.

In shade No. 3, a sub-roof, radiating to the animals, was cooled by evaporation of water. The upper roof was covered with sheet aluminum; the sub-roof, made of burlap sacks, was supported on 2- x 2-inch rafters about 3 feet below the aluminum roof. It had an area 21 x 21 feet as compared with 16 x 24 feet for the upper roof. The burlap was kept moist by 4 sprinklers spaced evenly above it, but below the aluminum roof. Surplus water dripped from the burlap to a concrete floor, which was drained through an outlet in one corner. The cattle were usually somewhat damp from these drippings (figure 2).

Figure 2. Double roofed shade (burlap and aluminum), burlap kept wet by sprinklers.

Figure 3. Double roof shade (hay and galvanized iron), lower roof wet.

Shade No. 4 also had two roofs, the lower likewise cooled by evaporation of water. The upper roof was of hay. The lower—sloping in shape—was of galvanized iron, placed about 3 feet below the hay. Four roof sprinklers kept the sub-roof uniformly wet. An eave trough carried the surplus water to a drain so that the floor was not wet (figure 3).

The four shades, each in a separate pen, were studied as a group. A large, aluminum "work" shade, over a scale, chute, and work alley, was

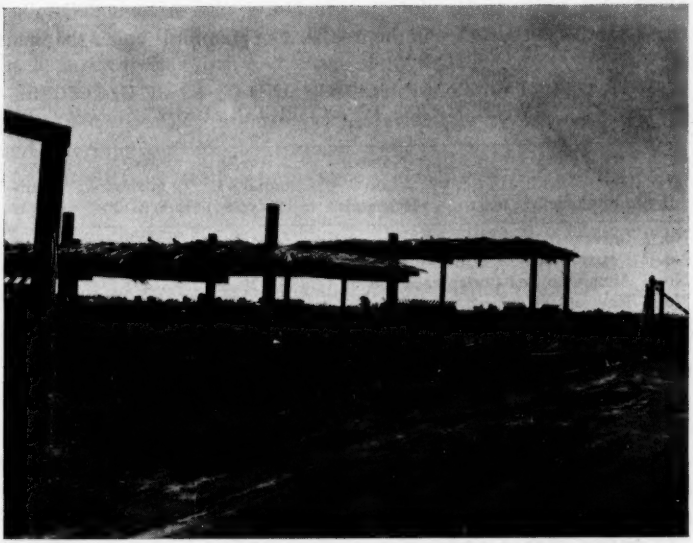

Figure 4. Hay covered shades, one 7-feet and the other 12-feet high.

adjacent to the four shades just described and during the hot part of the day cast a shadow into the pen of shade No. 4.

The sprinklers and desert cooler were generally started about 9:00 a.m. and turned off about 5:00 p.m., but at times were allowed to run until later in the evening.

Shades No. 5 and 6, which were in a different corral, were identical except for height, No. 5 being 7 feet high to the underside of the roof, and No. 6, 12 feet high. Both were 18 x 36 feet and were covered with hay supported by wire (figure 4).

Twenty-two good quality Hereford steers and 8 good Braford steers were divided, on July 28, among the four pens of shades. Throughout the test period, these animals were fed all the good alfalfa they would

eat, and during the last 27 days, received in addition, one pound of barley per head daily.

The data on weights and gains are shown in table 1. Two Brafords were assigned to each shade; all gained at approximately the same rate. Their greater heat tolerance apparently made them more insensitive to differences between shades. Inclusion of these animals made it impossible to ascertain the feed consumption of the Herefords. The weight-increase data in the table, therefore, is for the Herefords only. During the test, one Hereford in pen No. 4 died from undetermined causes, and is not included in the results. The Herefords under the shade

TABLE 1. COMPARISON OF WEIGHTS AND GAINS OF HEREFORDS WITH ACCESS TO ARTIFICIAL SHADE

Type of shade	Number of Herefords	Ave.[1] initial wt. July 28	Ave.[1] final wt. Sept. 20	Ave. daily gain
No. 1 Galvanized-iron roof	5	430	467	0.69
No. 2 Desert cooler	5	428	485	1.05
No. 3 Aluminum and burlap roof	6	429	472	0.80
No. 4 Hay and galvanized-iron roof	5*	420	468	0.89

* One Hereford died.

[1] Average weight and gains are only for the Herefords since there was no significant difference between the Brafords.

with evaporative cooler (No. 2) gained at the most rapid rate—1.05 pound per head daily—and those under the plain galvanized iron shade (No. 1) the least—0.69 pounds per head daily. The animals under the two shades with cooled surfaces gained at intermediate rates—0.80 pounds for shade No. 3 and 0.89 pounds for shade No. 4, per head daily.

Shortly after the evaporative cooler in pen No. 2 was started in the morning, all of the animals would lie near the fan, leaving only occasionally to eat and drink, usually between 1:00 and 2:00 p.m. During the late afternoon—except on extremely hot days—the cattle preferred to lie in the shade outside the walls of Shade No. 2, where they were exposed to the sky. The animals in this pen appeared to be more comfortable than those under the other shades and their rates of gain were a little higher.

Drip from the wet burlap roof of shade No. 3 kept the animals damp. They seemed to be almost as comfortable as those under the shade with the desert cooler (No. 2), and were more comfortable than those in the other two pens. One of the smaller Herefords in the pen with shade

No. 3 scoured during most of the test period and lost 15 pounds. If this animal is omitted from the calculations, the rate of gain for the steers under shade No. 3 becomes 1.02 pounds per day as compared to 1.05 pounds per day for those in the pen with the desert cooler (No. 2). Sanitation under this shade was a problem, however. The concrete floor had to be cleaned several times a week. Whenever the cattle became dry, they returned to the dripping burlap until damp again.

During the first part of the test period, the animals having access both to the double-roofed shade (No. 4) and to part of the large center workshade in the corral spent all of their time under the former. Later in the summer they used each shade about equally.

The animals under the plain galvanized iron shade (No. 1) suffered more than those in any other pen, as evidenced by increased panting and drivelling and by smaller gains.

Although body temperatures were not obtained because the steers were too wild, some respiration counts were made. The cattle using the desert cooler (No. 2) averaged 90 respirations per minute while outside their shelter, between 1 and 2 p.m. During the first half hour after their return to the cool shade, the respiration rates decreased to about 80 per minute. When the sprinklers on Shades Nos. 3 and 4 were not started until noon, respiration rates of the cattle averaged about 90 per minute. Those under the hay roof with sub-roof of wet galvanized iron (No. 4) showed little change in respiration rate after the sprinklers were turned on. When the animals under shade No. 3 became damp from the dripping burlap roof, an average drop of 19 respirations per minute occurred. If the sprinklers were run all day, cattle under the dripping burlap ranged 20 to 30 respirations lower than the other groups. On a particularly hot day when the air temperature at 3:00 p.m. was 118° F. and the relative humidity 15 percent, the average respiration rates under the various shades were: galvanized iron, 116; desert cooler, 107; double roof with galvanized iron, 105; and double roof with burlap, 80. Brafords usually had about as many respirations per minute as the Herefords. On this very hot day they averaged a few respirations more per minute than the Herefords, but their breathing was much shallower and the degree of their suffering was less.

In August and September, comparisons of shades were made on the bases of (a) air temperature beneath the shades, (b) temperature of roof surfaces next to the cattle, (c) temperature of ground beneath the shades, and (d) amount of solar, sky and ground radiation cut off by the shades. The average figures from a series of readings on a typical

day, which had a maximum air temperature of 109° and a minimum of 84° F. are given in table 2.

The air at the fan outlet from the desert cooler was about 20° F. lower than outside air temperature but quickly mixed with the warm air to give a temperature only 7° F. lower (table 2).

The temperatures of the under side of the roof surfaces were markedly different. The wet, shielded, galvanized-iron shade (No. 4) at times was 36° cooler, and the wet burlap (No. 3) was 45° cooler than the uncooled galvanized-iron roof.

TABLE 2. AVERAGE ENVIRONMENTAL CONDITIONS UNDER SHADES AT EL CENTRO, CALIFORNIA, ON AUGUST 6, 1948 BETWEEN 11:00 A.M. AND 1:00 P.M.

Comparison	No. 1 Galvanized-iron check shade	No. 2 Air cooled by desert cooler	No. 3 Burlap roof cooled by sprinklers	No. 4 Galvanized iron cooled by sprinklers
Average outside air temperature (°F)	103.5	103.5	103.5	103.5
Air temperature under shade (°F)	104.0	96.5	101.5	101.5
Temperature of underside of roof (°F)	127.5	99.5	89.0	92.0
Temperature of ground in shadow (°F)	107.5	95.3	97.2	100.0
Radiation from sun and sky cut off by shade (%)	56	—	61	62
Lowering of ground radiation by shade (%)	27	—	37	31

For the several shades energy coming from upper and lower hemispheres was measured with a hemispherical radiometer, which integrates the total energy falling on a horizontal surface. Pointed upward, the instrument measured the energy from the under side of the shade and from the unobstructed sky. Pointed downward, it indicated the energy reaching it from the comparatively cool ground in the shadow as well as from the hot unshaded ground away from the shade. The radiometer was maintained 18 inches above ground at the center of the shadow. This height approximates the center of a cow when lying down.

When the shade area was ample in relation to animal numbers, definite preference was shown for the high shade (No. 6) over the low (No. 5). As measured with the hemispherical total-energy radiometer, the high shade cut off 64 percent of the total solar and sky

radiation at noon, as compared to 61 percent for the low shade. Air temperature under both shades was about the same.

1949 Results

In 1949 a louvered shade (No. 7) was compared with the galvanized-iron check shade (No. 1) and the hay-covered shade with sub-roof of galvanized iron (No. 4). No water was used for cooling. The louvered shade was constructed of 1- x 10-inch boards set at such an

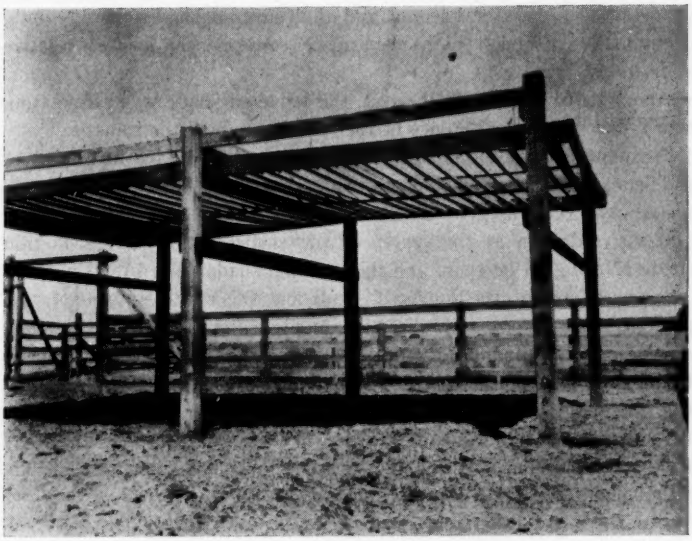

Figure 5. Louver shade.

angle that a solid shadow was cast upon the ground but allowed almost total exposure of the animals to the colder north sky. The angle and spacing of the slats were calculated for the latitude of El Centro, at noon of the longest day. The overall size of this shade was 13½ x 22 feet. At first this shade was 6 feet above the ground, but later this distance was increased to 9 feet. All three shades (Nos. 1, 4, and 7) were in the same corral, so that the cattle had access to all. (Shade No. 7 is shown in figure 5).

Animal preference was used as an indicator of the effectiveness of the shades. The test animals included eight Hereford and eight Braford

steers, averaging about 650 pounds each. They had access to pasture and, in addition, were fed an average of 6.4 pounds of good alfalfa per head daily. The Herefords made an average daily gain during the test period (66 days, from July 12 to September 16) of 1.20 pounds, while the Brafords averaged 2.13 pounds.

The cattle came in from pasture about 7 a.m. and went out again between 4 and 5 p.m. Gates of the corrals were left open, giving all animals free choice of the shades. The preferred resting place of the animals was the double-roofed shade (No. 4). The galvanized-iron shade (No. 1) was never used and the louvered shade (No. 7) only occasionally, although both were more conveniently located relative to feed.

About halfway through the test, the louvered shade was raised from 6 to 9 feet but the preference of the steers remained the same.

A comparison of the energy incident on a flat surface by means of the hemispherical radiometer was made at noon of August 24 when the air temperature was 106° F. Shade No. 4 cut out 58 percent of solar and sky radiation at the center of the shadow; the galvanized iron shade (No. 1), 54 percent; and the louvered shade (No. 7), 53 percent. The south end of the louvered shade cut off even less energy (51 percent). By the use of a directional radiometer, traversing from the north horizon through the zenith down to the south horizon, it was found that the high intensity radiation was energy reflected from the *top* side of the louvers down onto the animals. A person standing under the shade could feel the concentration of energy on the face and body from the north side. Raising the shade to 9 feet gave the same effect, although intensity was less at cow level.

For the 1949 experiment, the corral containing the low and high shades (Nos. 5 and 6) was reconstructed. The exit to the pastures was made equi-distant from the two shades and a water tank was placed in line with each. A manger for hay was set up between the two, so that it did not cast a shadow in either shaded area. The high and low shades were thus equally convenient. Eight Hereford steers averaging 650 pounds used these shades. They were fed about 13 pounds of alfalfa hay per head daily and gained 1.57 pounds per head daily during the test period (July 12 to September 16). The mean maximum temperature was 104° and the mean minimum 73° F. The high shade was used almost exclusively.

With small thermocouples and a recording potentiometer, air temperatures were measured for a 48-hour period at 1, 3, 6, 9, and 11 feet above the ground under the high and low shades. The effect of shade

height upon the air temperature at animal level proved negligible. Thus the main benefit to be derived from a shade was lowering the radiant heat load on the animals standing in the shadow. Calculations showed that the heat load on animals by radiation was 8 to 10 Btu/hr. lower per square foot of skin area under the high shade (12 feet) than under the low one (7 feet). The high shade gave the cattle greater exposure to the relatively cold sky.

A 10-foot high hay shade (No. 8) similar to Nos. 5 and 6 was constructed, but was not tested with instruments. Animals kept under it seemed as comfortable as those under the 12-foot shade. For general use, a 10-foot shade may be a little more practical than a 12 foot one.

Summary

The investigations show conclusively that the principal function of shade is to reduce the heat intake of the animals incident to the absorption of radiant energy from the sun. Air temperatures were about the same for all types of shades that did not make use of evaporative coolers.

In the order of preference and well-being of the cattle, the shades can be ranked as follows: (1) desert or evaporative cooler (Shade No. 2); (2) wetted burlap or galvanized iron roofs (Shades No. 3 and 4); (3) double roofed shade without spray cooling (No. 4, 1949); (4) hay covered shades, 12 10, and 8 feet high (Shades No. 6, 8, and 5), the highest shade being the best; and (5) plain galvanized iron roof and louvered shades (Shades No. 1 and 7).

The evaporative cooler gave the animals some relief from high temperatures and reduced the radiation heat load considerably, but the cost of constructing the three side walls and installing the motor driven fan was high. Also, the humidity during part of the summer tended to reduce the effectiveness of this type of cooling system. Sub-roofs with water sprays reduced the heat load but produced a definite sanitation problem. The galvanized iron shade (No. 1) reached a higher temperature and re-radiated more heat to the animals beneath it than any other material tested. A layer of hay over galvanized iron reduced the amount of radiant heat considerably.

Because of their greater exposure to the cool sky animals under high shades (10 to 12 feet high) received less radiant heat than those under low shades (7 feet high). Shades higher than 12 feet have the disadvantage of a fast moving shadow, necessitating the movement of cattle to heated ground.

For practical use in areas of low rainfall, the cheaper hay covered shades are adequate. Some hay covered shades constructed in the Imperial Valley of California are still standing after four years, with no repairs. Since the hay becomes heavy when wet by rain, the framework must be of fairly sturdy construction. In areas with heavier rainfall, aluminum or galvanized iron shades over a layer of hay will provide cool shade in the summer and protection from rain in winter.

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THE INFLUENCE OF TYPE ON FEEDLOT PERFORMANCE AND KILLING QUALITIES OF HEREFORD STEERS

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THE gaining-and-fattening ability of the "Regular" and "Comprest" types of Hereford steers has been a question in the minds of steer feeders for some time. In recent times the winning of fat stock shows by "Comprest" Herefords has renewed the widespread interest in type and its relation to feed utilization and the market value of animals.

Lush (1932) found that the correlations between measurements of feeder steers and subsequent gains, dressing percentages, and values of the dressed beef are low but statistically significant.

The most important measurements for high dressing percent and meat value are a large heart girth in connection with a shallow chest, a wide loin and large flank girth, a large initial weight, small paunch girth, head narrow at the eyes, and short height over hips.

While working with large and small type Hereford cows under range conditions, Woodward *et al.* (1942) found that feeder calves from the large type cows averaged heavier into the feedlot than did those from the smaller type. These calves also sold for more per head out of the feedlot and returned a slightly greater margin per steer.

Hultz (1927) reported little difference in economy of production between very low-set and very rangy Hereford calves. When calves of the same quality were compared, the rangy type was equal or superior to the low-set type in total gain, dressing percent and carcass grades.

Digestion balance trials conducted by Washburn *et al.* (1948) indicated no appreciable difference between the "Conventional" and "Compact" type Shorthorn steers with respect to feed capacity per unit of body weight and their ability to digest nutrients. Rib and round cuts of "Conventional" type carcasses carried more fat, while those of the "Compact" type had heavier bones.

The purpose of this study was to obtain additional information on type in Hereford cattle. Data are presented in this paper on the rate

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of gain, efficiency of gain, and carcass composition of "Comprest" and "Regular" type Hereford steer calves.

Materials and Methods

All the animals used in this study were obtained from the same ranch in November, 1948. The calves selected as "Comprest" were out of "Regular" type cows by bulls of "Comprest" type. Those calves of the "Regular" type were selected as being average in feeder grade of

Figure 1. Representative steers of the "Comprest" (right) and "Regular" (left) type used in this study.

calves produced on this ranch. They were out of cows of "Regular" type and were sired by bulls of the same type. Representative animals of the two types are shown in figure 1. The "Regular" type calves were taller at the shoulders, longer of body, greater in depth of chest, and greater in distance from chest floor to ground than were the "Comprest." Calves referred to in this experiment as "Comprest" had an average chest-height ratio of 1.683, while those classed as "Regular" type had a ratio of 1.579.

Following a 42-day preliminary feeding period, seven "Comprest" and seven "Regular" type calves were selected from their respective groups for use in this trial. A 285-day feeding period was used in this

test. During the first 112 days the calves were given a feed mixture computed to promote normal growth. In the remaining 173 days, they received a fattening ration containing about 59 percent concentrates. Both types received the same feed mixture *ad libitum* at all times. They were group fed according to type for the first 145 days of the test. At the end of this time they were placed in individual pens and finished.

TABLE 1. FEEDLOT PERFORMANCE OF "REGULAR" AND "COMPREST" TYPE CALVES DURING A 285 DAY FEEDING PERIOD

Comparison	"Regular"	"Comprest"
Number of animals	7	7
Gain in Weight		
Av. initial wt., lbs.	470	478
Av. final wt., lbs.	1000*	954
Gain per animal, lbs.	530*	476
Daily gain per animal, lb.	1.86*	1.67
Daily Feed Consumption		
41% cottonseed cake, lbs.	1.26	1.19
Ground hegari grain, lbs.	7.57	7.07
Alfalfa hay, lbs.	5.31	5.00
Cottonseed hulls, lbs.	5.81	5.50
Feed per Hundred Pounds of Gain		
41% cottonseed cake, lbs.	68	71
Ground hegari grain, lbs.	407	423
Alfalfa hay, lbs.	285	299
Cottonseed hulls, lbs.	312	329
T.D.N., lbs.	645	674

* Difference significant on .05 level.

The calves were slaughtered in the Meats Laboratory of The A. & M. College of Texas. Following slaughter, the right side of each carcass was sold. The left side was cut into wholesale cuts and the percentages of these cuts determined. A sample was obtained from the rib cut of the left side of each carcass and the percentage fat, lean, and bone determined by physical separation. These percentages were used to estimate the percent of these components in the whole dressed carcass as suggested by Hankins and Howe (1946).

Results and Discussion

Data concerning weight gains, feed consumption, and feed efficiency are shown in table 1. The difference of 46 pounds per head total feedlot

gain in favor of the "Regular" type was great enough to be statistically significant. Accumulated weights are shown in figure 2. It was observed that the two types of steers gained at approximately the same rate during the growth phase and for the first 60 days of the fattening phase. At this time the "Comprest" steers began to decline in rate of gain. Individual feed consumption data were collected for the last 140 days of this trial. By using covariance analysis it was possible to compare gains made by the two groups of steers during this period on

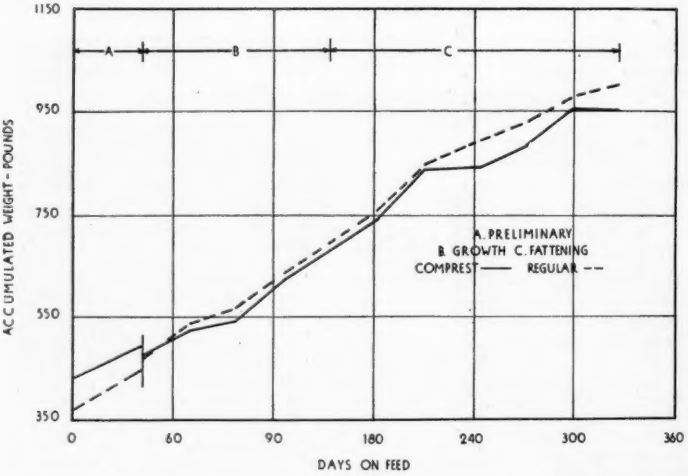

Figure 2. Average accumulated weight of "Comprest" and "Regular" type steers.

the basis of a common feed intake. When studied in this manner, the difference in gains made during this period was highly significant in favor of the "Regular" steers. The "Comprest" calves appeared to be more mature in conformation during the early part of the test than did those of the "Regular" type. After having been on the fattening ration for about 112 days, the "Comprest" steers appeared to be finished. The "Regular" type steers did not appear finished until they had been fed a fattening ration for about 173 days.

The steers consumed approximately 3.5 pounds of feed per hundred pounds of liveweight (figure 3) when they weighed about 500 pounds. This figure declined more rapidly for the "Comprest" steers than for the others as the feeding period advanced. When finished at weights of 950–1000 pounds, the steers were consuming between 1.5–2.0 pounds of feed per hundred pounds of liveweight daily.

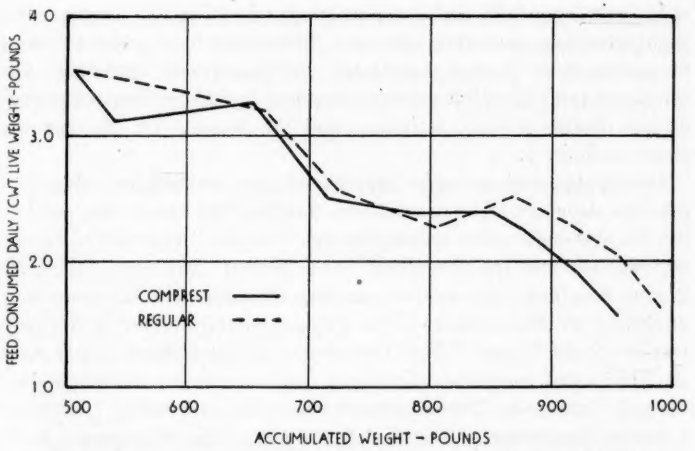

Figure 3. Average daily feed consumption per cwt. body weight of "Comprest" and "Regular" type steers.

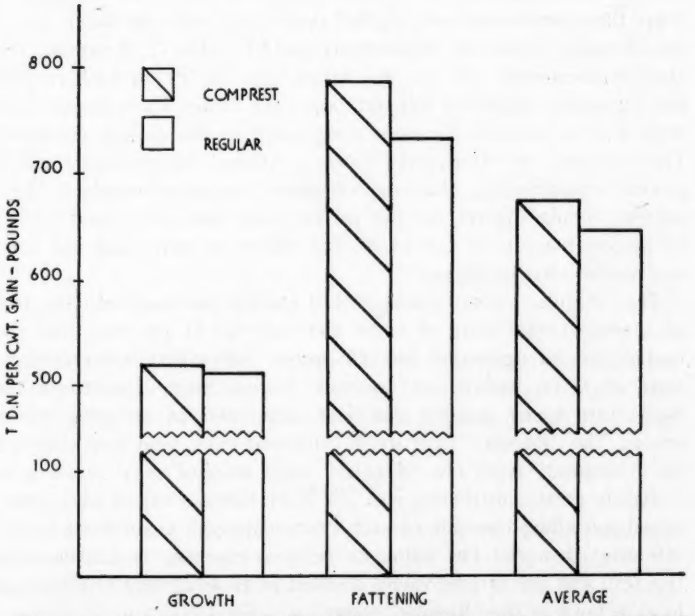

Figure 4. Calculated total digestible nutrients required per hundred pounds of gain by "Comprest" and "Regular" type steers.

In both the growth and fattening phase, the "Regular" steers had a slight advantage in feeding efficiency. These data for the last 140 days of feeding were studied statistically and found not significant. The calculated total digestible nutrients required to make a hundred pounds of gain during growth, fattening, and the average for the test are shown in figure 4.

Dressing percentages were determined from liveweights taken just prior to slaughter and warm carcass weights. The steers were held in dry lot over-night prior to slaughtering. "Regular" type steers yielded 64.9 percent and the "Comprest" 64.6 percent. This small difference is even less than expected for sampling variation. The carcasses were graded by an official grader of the Production and Marketing Administration of the United States Department of Agriculture. There were six Choice and one Good "Comprest" and four choice and three good "Regular" carcasses. The untrimmed head, hide, and shanks constituted a greater percentage of the final liveweight of the "Comprest" steers than they did of the "Regular" steers.

After aging for a period of 12 days the left side of each carcass was cut into wholesale cuts and the percentages of these cuts determined. When these percentages were studied statistically, only the shank cut of the "Regular" steers was significantly greater (table 2). A sample, the ninth-tenth-eleventh rib cut, was taken from the rib of each carcass and physically separated into fat, lean, and bone. These percentages were used to estimate the same components in the dressed carcasses. The carcasses of "Comprest" steers contained an estimated 35.51 percent separable fat, while the "Regular" carcasses contained 35.81 percent. Similar figures for the percent lean were 54.16 and 52.76; for percent bone, 12.61 and 13.39. The differences were small and none was statistically significant.

The "Regular" steers averaged 470 pounds per head initially, had an average feeder score of 15.14 and cost $24.71 per cwt. into the feedlot. Similar figures for the "Comprest" steers were 478 pounds, a score of 11.14, and $25.81 per cwt. United States Department of Agriculture feeder grading standards were used in assigning feeder grades. The "Regular" type steers consumed more total feed than did the "Comprest" type. The "Regular" steers weighed more, resulting in a slightly greater marketing cost per head. Carcass values were determined by selling one side of each carcass through commercial wholesale meat channels. The difference between expenses, as calculated in this test, and the carcass values resulted in an advantage of $8.42 per head in favor of the "Regular" steers.

"Regular" type steers made higher gains on less feed than "Comprest" steers in this study, although the difference in feeding efficiency was not significant. The "Comprest" steers slowed down in their rate of gain after having been on the fattening ration for about 60 days. At that time they had attained a greater percentage of their final feedlot

TABLE 2. SLAUGHTER DATA OF "REGULAR" AND "COMPREST" TYPE STEERS

Comparison	"Regular"	"Comprest"
Number of animals	7	7
Carcass, percent	64.90	64.60
Untrimmed hide, head, and shanks, percent	11.47	12.69
Carcass grades, U. S. Government		
High Choice	2	4
Middle Choice	—	1
Low Choice	2	1
High Good	3	1
Percentages of Wholesale Cuts		
Rib	8.51	8.14
Plate (short)	9.21	8.97
Chuck	26.18	26.26
Shank	3.69*	3.34
Brisket	4.99	5.53
Flank	6.44	6.30
Loin (full)	17.98	17.75
Rump	4.46	4.88
Round	18.54	18.83
Estimated Percent Separable Carcass Fat, Lean, and Bone		
Fat	35.81	35.51
Lean	52.76	54.16
Bone	13.39	12.61

* Difference significant on the .05 level.

weight than the "Regular" steers. This is interpreted as indicating earlier maturity in the "Comprest" steers. Their lower rate of gain may be explained by their decline in feed consumption per unit of body weight.

Summary

"Comprest" and "Regular" type Hereford steer calves from the same ranch have been compared as to rate and efficiency of gain under feedlot conditions. These calves were self-fed for a period of 112 days on a feed mixture calculated to promote normal growth. Following this,

they received individually a feed mixture containing about 59 percent concentrates for 173 days.

The "Regular" type calves were taller at the shoulders, longer of body, greater in depth of chest, and greater in distance from chest floor to ground than were the "Comprest."

The percent of market weight composed of untrimmed hide, untrimmed head, and shanks was greater in the case of the "Comprest" type steers.

More total and daily feedlot gain was made by calves of the "Regular" type.

There was a slight, but nonsignificant, difference in efficiency of feed utilization in favor of the "Regular" type calves.

Of the percentages of wholesale cuts studied, only the shank was significantly different. The percentage of this cut from the "Regular" type calves was greater.

The estimated percentages of separable fat, lean, and bone were not significantly different in carcasses from the two types.

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FACTORS INFLUENCING THE *IN VITRO* DIGESTION OF CELLULOSE BY RUMEN LIQUOR IN THE PRESENCE OF AN ANTISEPTIC

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HERBIVOROUS animals are capable of digesting cellulose by virtue of the enzymes produced by cellulolytic microorganisms which they harbor in either their rumen or caecum, and often, as in ruminants, in both. This microbiological hydrolysis of cellulose may be studied *in vitro* in at least two ways, either directly, by determining the amount of cellulose digested, or indirectly, by the quantitative determination of reducing substances which accumulate during digestion in the presence of an antiseptic.

A review of the literature shows that the general opinion is that cellulose is first broken down to the reducing substances, cellobiose and glucose, which are further changed to acids, alcohols and gaseous products (Norman and Fuller, 1942). In rumen liquor different organisms may be responsible for many of these changes, since, in the rumen, digestion occurs in the presence of mixed cultures of bacteria. An antiseptic such as toluene seems to favor the production of reducing sugars, as was first demonstrated in rumen liquor by Woodman and Evans (1938). Using this technique it was proposed to investigate further what factors, particularly dialyzable ones, affect the *in vitro* digestion of cellulose by rumen liquor in the hope that the knowledge gained might be of use in understanding and accelerating the *in vivo* process.

Experimental Methods

Rumen liquor was obtained from freshly slaughtered steers or cows, or from live steers by use of a large-bore stomach tube. The liquor was filtered through four thicknesses of No. 20 cheesecloth and placed in thermos bottles containing toluene, prior to use.

A mixture of 0.067 M Na_2HPO_4 and KH_2PO_4 was used as a buffer to give the desired pH which in most experiments was 6.98. As a source of cellulose, filter paper (S. and S., No. 589, Blue Ribbon) was used. It was ground through a 60 mesh screen in a Wiley mill. For some

experiments a suspension was prepared by grinding filter paper with water in a ball mill for 72 hours.

After testing various antiseptics, toluene was selected as most suitable. In some experiments the cultures were made 0.01 M with sodium fluoride.

Cultures were prepared in 250 ml. Erlenmeyer flasks by adding a definite amount of rumen liquor to a medium consisting of buffer, cellulose and antiseptic. The cultures were then incubated in a thermo-

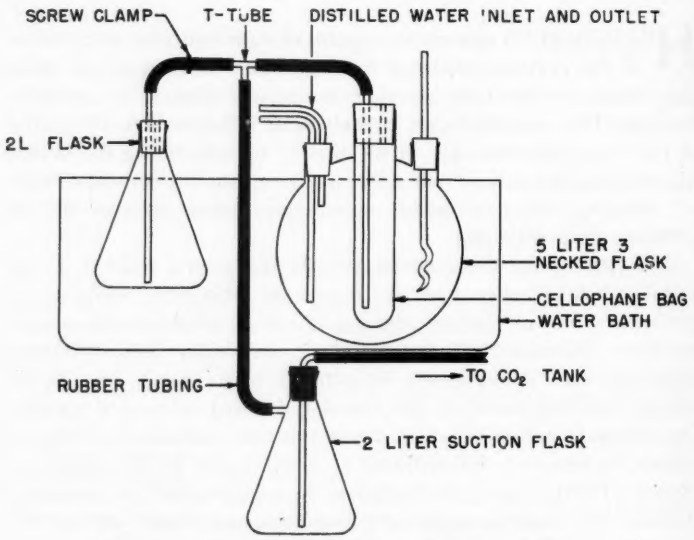

Figure 1. Apparatus for dialysis of rumen liquor.

statically controlled incubator at 39–40° C. for at least 72 hours. A typical culture had the following composition:

Rumen liquor	25 ml.
Cellulose	200 mg.
Buffer	95 ml.
Toluene	10 ml.

The Hagedorn and Jensen method (1923) was used for the determination of total reducing substances. Deproteinization was effected by the procedure of Doak (1939) using cadmium hydroxide. Reducing substances were finally calculated as glucose from the conversion

table given in Hawk, Oser and Summerson (1947). All values of glucose were expressed in milligrams per milliliter of culture, after correction for dilution and blank values.

In some experiments dialyzed rumen liquor was employed. This was prepared by using a dialyzing bag of cellophane (No. C seamless, Central Scientific Co.). Dialysis was carried out in a constant temperature bath at 40° C. and in the presence of carbon dioxide. The apparatus shown in figure 1 was used to obtain samples of dialyzed rumen liquor.

TABLE 1. DATA SHOWING ACCUMULATION OF REDUCING SUBSTANCES IN PRESENCE AND ABSENCE OF CELLULOSE AND TOLUENE

Culture	No. of cultures	Cellulose	Toluene	Glucose per ml. in culture after the following no. hours			Glucose Increase
				0	68	120	
				mg	mg	mg	mg/ml
"a"	2	+[1]	+	.059	.231	.231	.172
"b"	2	−[1]	+	.067	.170	.177	.110
"c"	2	+	−	.053	.036	.045	−.008
"d"	2	−	−	.053	.062	.069	.016

[1] +=present, −=absent.

Experimental Results and Discussion

Proof of Cellulose Digestion

To prove that accumulation of reducing substances in rumen liquor cultures containing toluene is due primarily to digestion of cellulose, it is necessary to show that there is more accumulation of these substances in the presence than in the absence of cellulose. Table 1 indicates the results obtained. It is evident that cultures as "a" which contained cellulose showed a considerable increase in reducing substances measured as glucose (0.062 mg.) over cultures as "b" which did not contain cellulose. The reducing substances found in "b" probably represent digestion of residual cellulose in the rumen liquor as well as digestion of other carbohydrates which happened to be present.

Cultures "c" and "d" were similar to cultures "a" and "b" except for the omission of the toluene. As is apparent in table 1, there is no

significant accumulation of reducing substances in the absence of toluene, hence the importance of adding this antiseptic.

By use of paper chromatography, the reducing substances which accumulate in cultures of rumen liquor were found to consist of three

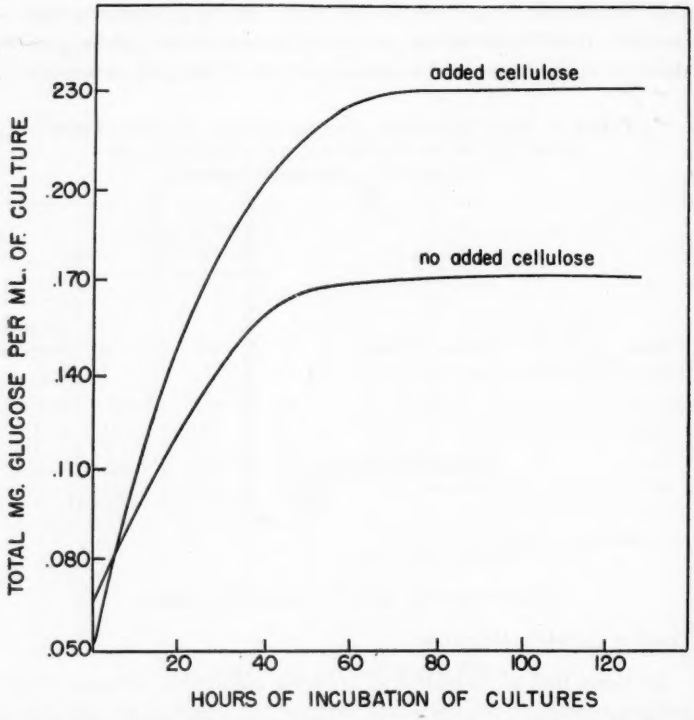

Figure 2. Accumulation of reducing substances in buffered rumen liquor cultures in the presence of toluene.

compounds, one of which is glucose. The remaining two were not definitely identified.

Figure 2 illustrates the typical accumulation of reducing substances in buffered rumen liquor cultures in the presence of toluene. The accumulation ceases after about 48 hours of incubation whether or not cellulose is added. In two experiments, soluble starch was used in place of the cellulose. In these cases reducing substances continued to accumulate for 96 hours or longer. In other experiments using the same

conditions except for the carbohydrate added it was found that rumen liquor was capable of hydrolyzing lactose, maltose and cellobiose but not chitin.

Optimum in vitro Conditions for Cellulose Digestion

Optimum pH. Digestion of cellulose was measured using phosphate buffers in a series of pH values ranging from 4.53 to 8.30. Digestion was similar in the range 4.53 to 7.35. Above pH 7.35 digestion dropped rather sharply. Values below pH 4.53 were not tested.

Effect of Buffer Mixtures. A comparison of the accumulation of reducing substances in four buffers, each at pH 6.98, was made. The results indicated that phosphate and glycine buffers were more effective whereas borate and citrate buffers were somewhat less effective than distilled water controls. Phosphate does not appear to be essential to cellulose digestion because rumen liquor which was dialyzed 45 hours did not show a greater increase of reducing substances in phosphate buffer than in glycine buffer. This procedure, however, does not eliminate the possibility that phosphate may have been held adsorbed on particles in the rumen liquor.

Action of Antiseptic. Of the following substances, tested in various concentrations, none promoted a greater increase of reducing materials than toluene. Some completely inhibited cellulose digestion as sodium arsenate, sodium cyanide, mercuric chloride, and iodoform. Iodoacetic acid, iodoacetic acid plus toluene, sodium flouride, benzoic acid, formalin, sodium chloride, formalin plus toluene, toluene plus thymol, formalin plus toluene plus thymol, chloroform, chloroform plus toluene, sodium fluoride plus toluene and boric acid all permitted some accumulation of reducing substances to occur.

Effect of Amount of Rumen Liquor. While there are not significant differences in the total cellulose digestion obtained in cultures which have 50–1000 mg. of filter paper present, there are substantial differences when the quantity of rumen liquor is varied. Increased digestion is obtained when the amount of rumen liquor is raised from 10 to 50 ml. per culture. Rumen liquor in the presence of cellulose and toluene but without added buffer, does not cause any more digestion than that of cultures in which smaller amounts of it have been employed.

Effect of Stirring before and During the Digestion Period. Rumen liquor was stirred for as much as 30 minutes in air, in the absence of toluene, prior to the preparation of the cultures. No loss in cellulolytic activity occurred. This indicates that anaerobic conditions are not

essential for collection of rumen liquor samples for the demonstration of cellulolytic activity in the presence of toluene. Stirring the cultures during the incubation period does not cause a change in the amount of reducing substances produced.

Effect of Aging on Digestion. If rumen liquor is allowed to stand in the presence of toluene at 40° C., it loses practically all its capacity for cellulose digestion in about 48 hours. Rumen liquor in cultures also appears to lose its cellulose digesting activity in about the same time (see figure 2).

Attempts to Isolate Cellulolytic Factors from Rumen Liquor

When rumen liquor is centrifuged for as short a time as two minutes at 2000 RPM the deposit may be shown to possess a significant amount of cellulolytic activity. This may mean that plant enzymes contribute towards digestion but possibly it is the result of bacteria remaining attached to the surfaces of the cellulose. However, the centrifuged liquor also possesses cellulolytic activity.

When rumen liquor is filtered through a Berkfeld filter, the filtrate does not show significant cellulose digesting capacity. Possibly the enzyme concerned is not secreted into the medium but digestion of cellulose proceeds through a direct contact between the surface of the organism and the cellulose fiber. The above mentioned inactivation of rumen liquor on standing in the presence of toluene for 48 hours could well be due to a disruption of the bacterial surfaces.

Experiments with Dialyzed Rumen Liquor

Influence of Minerals. When rumen liquor is dialyzed for 18 hours against distilled water in the apparatus shown in figure 1, the addition of ash solutions of rumen liquor and ash of alfalfa hay cause increased amounts of reducing substances to accumulate in comparison with cultures which are not dialyzed, as shown in table 2. That minerals may be concerned in digestion of cellulose *in vitro* has already been indicated by the work of Burroughs *et al.* (1950). The identification of these possibly essential minerals could not be made. Addition of small quantities of ferrous sulfate or cobaltous nitrate did not stimulate digestion.

Influence of Organic Factors. Rumen liquor which has been dialyzed for 48 hours with the resulting loss of cellulose digesting capacity, could not be stimulated to digest cellulose once again by the addition of mixtures of various vitamins. Those used included Vitamin A, alpha

tocopherol, calciferol, 2-methyl naphthoquinone, thiamine chloride, nicotinic acid, riboflavin, pyroxidine hydrochloride and calcium pantothenate. Vitamin B_{12} also was without effect.

Yeast, alfalfa, and beef extracts as well as bovine blood, failed to stimulate cellulose digestion. The addition of the evaporated dialyzate was inhibitory to cellulose digestion. Boiled rumen liquor and rumen liquor passed through a Berkfeld filter were without effect.

TABLE 2. INFLUENCE OF MINERALS ON CELLULOSE DIGESTION

No. of cultures	Culture variable	Initial glucose	Glucose after 66 hours	Glucose Increase
(A) Undialyzed rumen liquor aged 18 hours				
		mg/ml	mg/ml	mg/ml
2	+cellulose	.102	.138	.036
2	—cellulose	.117	.114	—.003
2	10 ppm Fe++	.102	.125	.023
2	5 ppm Co++	.095	.122	.027
2	Ash of rumen liquor	.100	.127	.027
2	Ash of alfalfa hay	.095	.118	.023
(B) Rumen liquor dialyzed 18 hours				
2	+cellulose	.060	.087	.027
2	—cellulose	.060	.080	.020
3	10 ppm Fe++	.065	.089	.024
3	5 ppm Co++	.065	.091	.026
3	Ash of rumen liquor	.047	.100	.053
3	Ash of alfalfa hay	.037	.095	.058

Summary

Cellulose digestion may be demonstrated by the *in vitro* action of rumen liquor on filter paper in the presence of toluene, through the accumulation of reducing substances.

Toluene proved to be the most effective antiseptic to prevent further changes of the reducing substances which are first produced in cellulose digestion. The optimum pH lies between 4.53 and 7.35. Phosphate and glycine buffers appeared to be more favorable than borate or citrate. Anaerobic conditions are not essential. The cellulolytic activity of rumen liquor, with or without added buffer, decreases on standing in the presence of toluene, and ceases after about 48 hours.

The factors which promote digestion of cellulose by rumen liquor do not appear to pass through a Berkfeld filter.

The loss of cellulolytic activity of rumen liquor on dialysis cannot be restored by the addition of various vitamin mixtures, yeast, beef and alfalfa extracts, bovine blood, evaporated dialyzate, or boiled or filtered rumen liquor. Some of the activity may be restored by the addition of solutions of ashed alfalfa hay and of rumen liquor.

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SPERMATOZOAN MOTILITY AS A MEASURE OF SEMEN QUALITY[1]

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SINCE the first studies were made in spermatozoan physiology, the motility of spermatozoa has been utilized as an indicator of semen quality. Although many methods have been used for classifying semen on the basis of spermatozoan motility, all of these have been based on estimates. Attempts to correlate estimates of spermatozoan motility in semen from normal bulls with fertilizing capacity when used for artificial insemination have not been too successful. Lasley and Bogart (1943) found no correlation between motility rating (as estimated by observing a drop of semen under the low power objective of the microscope) and fertilizing capacity in 296 ejaculates used for the insemination of 2,118 cows. They suggested that possibly these results were due to the inaccuracy of the method used for rating motility.

In view of these results a search was made for a more accurate method of determining spermatozoan motility. Finally, the method of Brady and Gildow (1939) was selected. The data reported herein on spermatozoan motility are based on determinations by a modification of this method.

Materials and Methods

The semen used in these experiments was collected from 7 registered Hereford bulls located at the San Carlos Agency, San Carlos, Arizona. All ejaculates studied were used for the artificial insemination of range cows with the exception of a small portion which was used for experimental purposes.

The methods used for determining the percentages of live and resistant spermatozoa in the semen were described in detail by Lasley and Bogart (1943). Spermatozoan motility was determined by a modification of the hemacytometer method of Brady and Gildow (1939) and

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is described as follows: a small amount of semen was drawn into the diluting pipette and diluted 1 to 200 with a phosphate buffer solution.[3] After this diluted semen had been thoroughly mixed by shaking, a drop was placed on the counting chamber and covered with a clean cover slip. The counting chamber was then placed under the high power objective of the microscope, and the number of non-motile and weakly motile spermatozoa in 25 large squares counted and recorded. The counting chamber was then placed in the freezing compartment of a refrigerator. After the dilute suspension of spermatozoa under the cover slip had frozen, the counting chamber was removed from the refrigerator and the film thawed in direct sunlight. The total number of spermatozoa on the same 25 large squares were counted and recorded. The percentages of motile and progressively motile spermatozoa were calculated as follows:

$$\frac{\text{No. non-motile sperm. before freezing}}{\text{Total no. spermatozoa after freezing}} \times 100 = \text{Percent non-motile sperm.}$$

$$\frac{\text{No. weakly motile sperm. before freezing}}{\text{Total number sperm. after freezing}} \times 100 = \text{Percent weakly motile spermatozoa.}$$

The percentage of non-motile spermatozoa subtracted from 100 gave the percentage of motile spermatozoa. The percentage of progressively motile spermatozoa was determined by subtracting the percentage of non-motile plus the percentage of weakly motile spermatozoa from 100 percent.

Results

The Percentage of Motile and Progressively Motile Spermatozoa as Determined by the Hemacytometer Method

The method of Brady and Gildow (1939) for determining the percentage of motile spermatozoa in ram semen consisted of diluting a portion of the semen sample 1 to 100 with a physiological saline solution and then counting the number of dead cells in 16 squares of the hemacytometer. Another portion of the sample was diluted 1 to 100 with one percent alcohol and the total number of cells counted. The percentage of motile cells was determined by subtracting the non-motile cells in the first sample from the total number in the second alcohol-diluted sample. After considerable experimentation with this method, it was found that more accurate results could be obtained if a small

[3] If the semen had been diluted with egg yolk-buffer before the count was made, only the buffer solution was used for dilution. If the semen had not been diluted with egg yolk-buffer, a small amount of egg yolk was added to the buffer to keep the spermatozoa motile while the count was being made.

amount of egg yolk was used in the phosphate buffer diluting solution. The combination of phosphate buffer and egg yolk kept the spermatozoa motile while the count was being made. A second modification which also improved the accuracy of the method was the killing of the cells on the hemacytometer by freezing. When the film under the cover slip had thawed, the total number of cells on these same 25 squares were counted.

The results obtained by the use of this method are shown in table 1. In the 78 ejaculates studied, an average of 64.1 percent of the spermatozoa were motile. The range was between 35 and 86 percent. In these same ejaculates it was also found that an average of 51.6 percent of

TABLE 1. SHOWING THE PERCENTAGE OF MOTILE AND PROGRESSIVELY MOTILE SPERMATOZOA IN 78 EJACULATES OF BULL SEMEN BEFORE AND AFTER STORAGE

Item	Fresh Semen		Semen stored 4 days at 10–12° C.[1]	
	Mean	Range	Mean	Range
Percent motile spermatozoa	64.1	35–85	34.9	0–67
Percent progressively motile sperm	51.6	20–78	14.2	0–48

[1] In egg yolk-phosphate buffer solution.

the cells displayed strong, forward, progressive motion. The range here was between 20 and 78 percent. These figures are not in agreement with those of some other workers who have made estimates of as much as 100 percent motility. On the basis of the counts in this investigation, it would seem that most motility estimates found in previous publications are too high. This may be due to the fact that many of the inactive spermatozoa are moved about by the active ones, giving the false impression that nearly all of the cells are highly motile.

As can be seen in table 1, there was considerable decline in motility after a storage period of 4 days even in the presence of egg yolk. Although some samples contained no motile spermatozoa after storage, others withstood storage well and contained almost as large a percentage of motile cells as did some freshly ejaculated samples.

The Relationship Between the Percentages of Live and Motile Spermatozoa

The percentages of live and motile spermatozoa were determined in the same 78 ejaculates in order to study the relationship between these

two characteristics. The ejaculates always contained a larger percentage of live than motile cells. Nevertheless, there was a highly significant correlation between these two characteristics ($r = 0.614$). The percentages of progressively motile and live spermatozoa were also significantly correlated ($r = 0.464$). Unpublished data indicate that the correlation between these characteristics might be much higher in bull

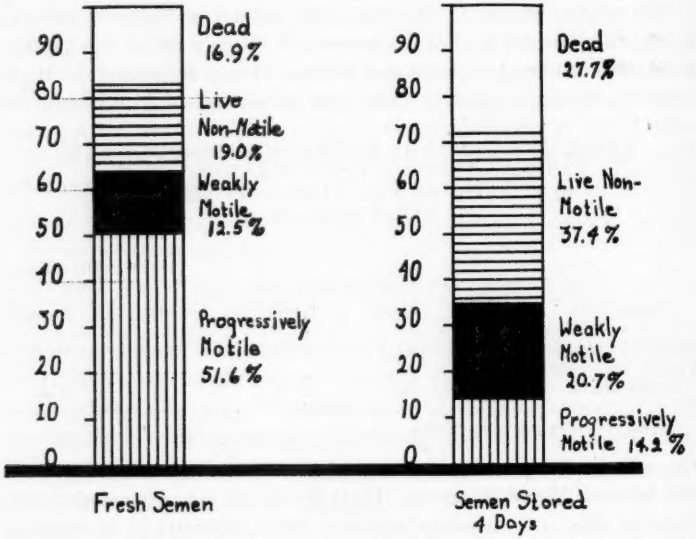

Figure 1. Showing the different types of spermatozoa in 78 samples of bull semen before and after storage (diluted with E. Y. B.)

epididymal spermatozoa since after activation with the proper diluent most of the live, non-motile cells became progressively motile.

The use of the hemacytometer method of determining spermatozoan motility and the opal blue-eosin staining method of determining the percentage of live spermatozoa in these same ejaculates revealed 4 different types of cells as shown in figure 1. The percentage of each of these types in fresh ejaculates were: (1) dead or stained, 16.9 percent, (2) live, non-motile, 19.0 percent, (3) weakly motile, 12.5 percent, and (4) progressively motile 51.6 percent. The last three types are included in the non-stained or live group. The difference between the percentages of live and motile spermatozoa represented group 2 or the live non-motile cells. Group 3 included those cells which contained all types of motion

other than progressive, forward motion. The oscillatory and rotatory motion described by Walton (1933) was included in this group. Group 4, included only those types which were progressively motile, and according to Walton this is the type of cell that takes part in fertilization.

The storage of these same ejaculates in egg yolk-phosphate buffer at a temperature of 10 to 12° C. for 4 days brought about a considerable change in the proportion of different types of spermatozoa as is shown in figure 1. Although an average of 72.3 percent of the cells in the ejaculates were still alive, a large percentage of these were non-motile. Of the total number of spermatozoa in the ejaculates, 27.7 percent were dead or stained, 37.4 percent were alive but non-motile, 20.7 were weakly motile and only 14.2 percent were progressively motile. Thus, the percentage of non-motile, live cells had increased almost two-fold, whereas those possessing progressive motility had decreased from 51.6 to 14.2 percent.

The Relationship Between the Percentages of Motile and Resistant Spermatozoa

The resistance test of Lasley and Bogart (1943) was developed as a method for testing semen quality because it was recognized that the percentage of live spermatozoa as determined by the opal blue-eosin staining method contained many non-motile as well as motile cells. It was assumed that the cold temperature shock killed many of the weak cells leaving only the stronger ones alive. Thus, a determination of the percentage of live spermatozoa after the cold temperature shock should give a better measurement of semen quality. It was thought that the resistant sperm (those surviving the cold shock in egg yolk phosphate buffer) would also be those which were progressively motile. In order to test this point, the percentages of resistant and progressively motile spermatozoa were determined in the same 63 ejaculates. These percentages were found to be almost identical. The mean percentage of resistant spermatozoa was 50.35 as compared to 51.09 for those showing progressive motility. However, variation was greater in the percentage of resistant spermatozoa (coefficient of variation of 36.98 percent) than in those showing progressive motility (coefficient of variation of 23.37 percent).

Although these characteristics were not always equal in the same ejaculate, there was considerable relationship between them. In calculating the coefficient of regression it was found that for each 10 percent increase in the number of progressively motile spermatozoa, there was

a corresponding increase of 5.44 percent in the percentage of resistant spermatozoa.

The Relationship Between the Percentages of Live and Motile Spermatozoa after Storage

The percentage of live, motile, and progressively motile spermatozoa in freshly ejaculated semen were all significantly correlated with the percentage of live spermatozoa in semen after a storage period of 4 days in egg yolk-buffer (table 2). However, the percentage of live spermatozoa in fresh semen was a better measure than the percentage of motile spermatozoa in predicting the percentage of live spermatozoa in semen after storage as shown by a higher coefficient of correlation. On the other hand, none of these characteristics was correlated with the percentage of motile or progressively motile spermatozoa after storage.

TABLE 2. THE RELATIONSHIP BETWEEN THE VARIOUS SEMEN CHARACTERISTICS BEFORE AND AFTER STORAGE (EACH FIGURE IS THE COEFFICIENT OF CORRELATION)

Item	Semen stored for 4 days in E.Y.—B.		
	Percentage live sperm.	Percentage motile sperm.	Percentage prog. motile sperm.
Percentage of live sperm. in fresh semen	0.788**	0.003	0.006
Percentage of motile sperm. in fresh semen	0.614**	0.012	—0.006
Percentage of prog. motile sperm. in fresh semen	0.464**	0.024	0.122

** Highly significant, $P<.01$.

The Relationship Between Spermatozoan Motility and Conception Rate in Range Cattle

One of the main uses of a method for evaluating semen is to determine if it is reliable in predicting the fertilizing capacity of semen. To test this point, the percentages of motile and progressively motile spermatozoa were determined by the hemacytometer method in 67 ejaculates of bull semen used for the artificial insemination of range cows. All semen was used for insemination purposes within 4 hours after it was collected. One cc. of semen diluted 1 to 4 with egg yolk-phosphate buffer was used for inseminating 740 cows. Fertility tests were based on rectal palpation of the cows between the 3rd and 6th

months of pregnancy, or on recurring estrus at the first regular heat period following insemination. An average of 11 cows were inseminated with semen from each diluted ejaculate, although this number varied between 1 and 25. Because of the fact that some ejaculates were used for the insemination of only a few cows, fertility estimates probably varied somewhat from the actual figure. The fertilizing capacity of the 67 ejaculates did vary considerably as shown by a coefficient of variation of 27.47 percent. A further statistical analysis of these data showed that there was a highly significant correlation between conception rate and the percentage of motile spermatozoa in the ejaculates ($r=0.314$). For each 10 percent increase in the percentage of motile spermatozoa, fertility increased by 4.6 percent.

In these same ejaculates there was no significant correlation between the percentage of progressively motile spermatozoa and conception rate ($r=0.167$). For each 10 percent increase in the percentage of progressively motile spermatozoa, there was a corresponding increase of only 2.1 percent in conception rate.

The low correlation between these characteristics and conception rate are to be expected because of the errors encountered in measuring the fertility of the ejaculates. Likewise, there was also some error in determining the percentage of motile and progressively motile spermatozoa in the ejaculates. Another contributing factor was that all of the semen used was collected from bulls of normal fertility and there was very little difference in the semen quality. Even if some ejaculates did contain only 20 to 30 percent motile spermatozoa, there were still millions of cells present in each portion of the ejaculate used for insemination which were potentially capable of uniting with the ova in the process of fertilization. In such a study one would not expect a semen sample which contained 2 million motile spermatozoa to settle twice as large a percentage of cows than would a sample containing only one million.

Summary

Results are presented in which the hemacytometer method was used for determining the percentages of motile and progressively motile spermatozoa in bull semen. In 78 ejaculates of fresh bull semen, an average of 64.1 percent of the spermatozoa were motile and 51.6 percent were progressively motile. These same ejaculates contained only 34.9 percent motile and 14.2 percent progressively motile spermatozoa after a storage period of 4 days at a temperature of 10 and 12° C. in egg yolk-phosphate buffer solution.

By using the opal blue-eosin staining method for determining the percentage of live spermatozoa and the hemacytometer method for determining the percentages of motile and progressively motile spermatozoa in the same ejaculates, four types of cells were observed in bull semen. These types were (1) dead or stained, (2) live, non-motile, (3) weakly motile and (4) progressively motile.

A highly significant correlation was found to exist between the percentages of live, motile and progressively motile spermatozoa in fresh semen. All of these characteristics were also significantly correlated with the percentage of live spermatozoa in semen after storage for 4 days in egg yolk-phosphate buffer. However, none of these characteristics were correlated with the percentage of motile spermatozoa after this same storage period.

The average percentages of resistant and progressively motile spermatozoa were almost identical when determined in the same 63 ejaculates although there was considerable variation in each of these characteristics. For each 10 percent increase in the percentage of progressively motile spermatozoa, there was a corresponding increase of 5.44 percent in the percentage of resistant spermatozoa.

The percentage of motile spermatozoa in 67 ejaculates of fresh semen was significantly correlated with fertilizing capacity although this correlation was low ($r=0.314$). The correlation between the percentage of progressively motile spermatozoa and fertilizing capacity was not statistically significant ($r=0.167$).

On the basis of the data reported in this study, it would seem that the determination of the percentage of motile spermatozoa in bull semen by means of the hemacytometer is a good measure of semen quality.

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THE DETERMINATION OF NUMBERS OF SPERMATOZOA IN BULL SEMEN BY MEASUREMENT OF LIGHT TRANSMISSION

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THE use of artificial insemination of dairy cattle has been increasing greatly and steadily in the United States. As a consequence, semen has been diluted at higher and higher levels. The dilution studies of Salisbury and Bratton (1948) and of Willett (1950) have demonstrated that spermatozoan numbers can be reduced only to certain limits without reducing breeding efficiency. When diluting semen at high levels in the field it is, therefore, necessary to have some means of determining spermatozoan numbers in semen quickly and accurately. Emik and Sidwell (1947) and Salisbury *et al.* (1943) have reviewed the literature concerning the determination of numbers of spermatozoa in semen by measuring turbidity. The latter have described a procedure for determining numbers of sperm in bull semen by means of a custom-built electric colorimeter. The writers have found that the ratio of semen to diluter as used by them is unsatisfactory, however, when used with standard instruments. The American Foundation for the Study of Genetics has developed a procedure which has proven to be quite accurate and easily duplicated and which makes use of an instrument available on the market. This procedure, as well as the results of some studies conducted during its development, is described below.

Experimental Procedure and Results

Study of Methods

Prior to the initiation of these experiments, a Cenco-Sheard-Sanford "Photelometer," industrial type B-2, had been used in this laboratory for determining spermatozoa in semen according to the procedure of Salisbury *et al.* (1943). The Photelometer measures the percentage of light transmitted through a liquid and has a scale with graduations from 0 to 100. These figures represent percentages of light transmission.

Since it is inexpensive, simple to operate and accurate, the authors

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decided to attempt to develop a procedure for use with this instrument which would enable the accurate measurement of spermatozoan numbers. Preliminary studies indicated that a dilution of 1:40 would give readings most of which would be within the most sensitive range of the instrument. This range is from 20 to 70% light transmission (Central Scientific Co., 1949). Later studies confirmed the selection of this ratio, for of 142 samples upon which readings were made only 7 gave readings below 20 and 4 above 70. Since Emik and Sidwell (1947) had concluded that a 4% chlorazene solution was more desirable than a sodium chloride solution or a phosphate buffer for diluting sheep semen prior to measuring turbidity to determine spermatozoan numbers, it was decided to compare this solution with a sodium citrate buffer, which was used by Salisbury *et al.* (1943) and which is commonly used in the field with egg yolk as an extender for semen. Since part of the error in determining spermatozoan numbers in semen by turbidity may be due to interfering substances in the seminal plasma, it was also decided to study the feasibility of removing a major portion of the plasma by centrifuging diluted semen, decanting and rediluting the spermatozoa. The experiment described below was, therefore, planned.

Five treatments were compared with semen from each of 27 ejaculates:

(1) One-tenth cc. of semen was placed in a 12 cc. graduated centrifuge tube. To this was added 4 cc. of 4% chlorazene solution. Following centrifuging to precipitate the spermatozoa, the supernatant liquid was removed and additional chlorazene solution added to bring the total volume of liquid to 4 cc. This volume is ample to enable accurate readings in the Photelometer. The mixture was stirred thoroughly by stoppering the tube and inverting it several times, and was then transferred to a standardized tubular absorption cell 17 mm. in diameter. These cells are of the type supplied with the Photelometer and have been developed for work of this nature by the manufacturer.

(2) One-tenth cc. of semen was placed in an absorption cell and 4 cc. of chlorazene solution was added. The mixture was stirred by stoppering and gently inverting the cell several times.

(3) Same as (1) above except that a 3.2% sodium citrate dihydrate solution was used in place of the chlorazene solution.

(4) Same as (2) above except that the sodium citrate solution was used.

(5) One-tenth cc. of semen was placed in a test tube approximately 9 mm. in diameter. One cc. of 3.2% sodium citrate solution was added. The liquid was mixed by inverting the tube several times. This is the procedure outlined by Salisbury *et al.* (1943).

Photelometer readings were made with the various mixtures in the containers as indicated. Prior to each determination an absorption cell, or small tube in the case of treatment 5, containing the respective diluter was placed in the instrument and the amount of light adjusted to bring the reading on the scale to 100. The green filter supplied with the Photelometer has been used in all the work reported in this paper. The number of spermatozoa in the semen from each ejaculate was counted by means of a haemacytometer. A Trenner automatic diluting pipette and 4% NaCl solution were used for diluting semen when making these counts. Spermatozoa in all of the 400 small squares of

TABLE 1. CORRELATION BETWEEN LOGARITHMS OF PHOTELOMETER READINGS WITH DIFFERENT SEMEN TREATMENTS AND HAEMACYTOMETER COUNTS. TWENTY-SEVEN SAMPLES WITH EACH TREATMENT

Semen Treatment	Correlation Coefficient
Centrifuged. Diluted 1:40 in chlorazene solution	—0.92
Not centrifuged. Diluted 1:40 in chlorazene solution	—0.96
Centrifuged. Diluted 1:40 in citrate solution	—0.95
Not centrifuged. Diluted 1:40 in citrate solution	—0.96
Not centrifuged. Diluted 1:10 in citrate solution	—0.93

the improved Neubauer chamber of the haemacytometer were counted. For each treatment the correlation between the logarithms of the Photelometer readings and the haemacytometer counts was determined. The transformation to logarithms made the data linear instead of curvilinear.

A summary of the results is presented in table 1. There are no significant differences between any of the correlation coefficients. It can be seen that the correlation coefficients for the centrifuging procedures are no higher than for those in which the semen was not centrifuged. Nothing was, therefore, gained by removing the major portion of the seminal plasma prior to determining the opacity of the spermatozoan suspension. Possibly the gain in accuracy brought about by removing the seminal plasma was lost due to the extra manipulation involved. The fifth treatment compared surprisingly well with the others, for in a previous study under field conditions using a similar procedure, a correlation coefficient of only —0.79 was obtained with 110 samples. It was concluded that the fourth treatment, namely the 1:40 dilution with 3.2% sodium citrate, was most practical under field conditions for diluting semen.

A trial with 13 samples has demonstrated that either a 2.8, 3.2, or

3.6% sodium citrate dihydrate solution may be used for diluting the semen for Photelometer readings without there being any significant difference in results.

Large-scale Trial

Haemacytometer counts and Photelometer readings using the fourth procedure outlined above were made with 142 ejaculates from 50 bulls in 3 different bull studs. These data were accumulated to establish accurately a regression line and correlation coefficients. A wide sampling of bulls was made to ascertain if there might be individual bulls whose

TABLE 2. CORRELATION AND REGRESSION COEFFICIENTS WITH SEMEN FROM BULLS IN THREE STUDS. SEMEN DILUTED 1:40 IN CITRATE SOLUTION

Stud	No. Samples	No. Bulls	Correlation Coefficients	Regression Coefficients
I	25	12	—0.93	—0.25
II	28	18	—0.97	—0.28
III	89	20	—0.97	—0.28
All studs	142	50	—0.97	—0.28

semen contained substances which would cause inaccurate Photelometer readings.

The haemacytometer counts ranged from 24,000,000 to 2,830,000,000 and averaged 1,249,000,000 spermatozoa per cc. The Photelometer readings ranged from 16 to 95 and averaged 40. Correlation and regression coefficients for the individual studs and for the 3 studs combined are presented in table 2. There are no statistically significant differences between the coefficients for the different studs. The overall correlation coefficient of —0.97 indicates that 94% of the variation in spermatozoan numbers as determined by the haemacytometer is accounted for by the Photelometer readings. The standard deviation from regression is 1.10 in terms of percentage light transmission. This figure represents the average vertical deviation from the regression line when percentages of light transmission form the ordinates, and is 2.7% of the mean Photelometer reading. The corresponding figure for spermatozoan numbers (the abscissas) is 151 million, which is 12% of the mean or 5% of the range.

The regression formula based on the data from the 142 ejaculates is as follows:

$$Y=1.576-0.276\ (X-1.249)$$

Here Y is the logarithm of the Photelometer reading and X is the number of spermatozoa (in billions) per cc. of semen. A table, calculated by means of this regression formula and giving the numbers of spermatozoa corresponding to the various readings on the Photelometer, has been computed by the authors and shall be supplied upon request.

An examination of the data from the individual bulls did not give any indication that any bull gave readings differing appreciably from the expected. To examine this possibility more thoroughly, within-bull correlation and regression coefficients were calculated for the 12 which had 4 or more samples. The correlation coefficients for individual bulls ranged from —0.72 to —0.99 and averaged —0.93. The regression coefficients ranged from —0.20 to —0.43 and averaged —0.29. There were no significant differences between any of these coefficients.

Accuracy and Sources of Error

Nine different Photelometers were compared to determine whether or not there were significant differences between instruments and whether or not each individual instrument would require calibrating. Light transmission readings were made with duplicate samples, each diluted 1:40 with a solution of 3.2% sodium citrate dihydrate, of five ejaculates from five different bulls. A total of 90 readings were thus made. The mean square for Photelometers was 6.39 when light transmission values were analyzed and 118.15 in terms of spermatozoan numbers as determined by means of the regression formula. These mean squares were very highly significant (probability $<0.01\%$). The mean square for within duplicate pairs was 1.38 (light transmission) or 2701 (spermatozoan numbers) with 45 degrees of freedom. There was no significant interaction between instruments and ejaculates.

The authors' Photelometer, which was used in obtaining the data for the calculation of the regression formula presented above, was one of the nine included in this test. The average reading for this instrument was almost exactly the same as the average for the nine. Only two of the eight instruments were significantly different from the authors', and these were barely so at the 5% level of probability. Individual readings on these eight instruments differed on the average from those made with comparable samples on the authors' Photelometer by 1.3 percentage light transmission or approximately 56 million spermatozoa per cc. of semen. The maximum deviation was 4 in terms of percentage light transmission or approximately 160 million spermatozoa. This average deviation of 1.3 is slightly greater than the standard deviation

from the regression line (1.10) discussed previously. Furthermore, the maximum deviation of 4 was within the confidence limits (probability $=0.05$) between Photelometers for single observations. On the basis of these data, it is concluded that the use of an uncalibrated instrument and the regression formula presented above would enable the determination of spermatozoan numbers accurately enough to meet the needs of a bull stud. For more precise work somewhat greater accuracy would be obtained with most instruments by calibration.

An additional study was made with 25 ejaculates from 20 bulls to determine the relative magnitudes of the different sources of variation

TABLE 3. SOURCES OF VARIATION WHEN COMPARING HAEMACYTOMETER COUNTS WITH SPERMATOZOAN NUMBERS ESTIMATED WITH THE PHOTELOMETER. DUPLICATE READINGS MADE WITH 25 EJACULATES FROM 20 BULLS

Source of variance	Degrees of freedom	Mean square
Photelometer counts within ejaculates	25	24.34
Haemacytometer counts within ejaculates	25	86.16
Interaction methods and ejaculates	24	261.36

when comparing haemacytometer counts with Photelometer readings when the semen is diluted 1:40 in sodium citrate solution and not centrifuged. Duplicate haemacytometer counts and Photelometer readings were made for each of the 25 ejaculates. A separate dilution was made for each of the duplicate haemacytometer counts. The number of spermatozoa were estimated for each Photelometer reading by using the table calculated by means of the regression formula determined from the data for the 142 ejaculates. Pertinent data from the analysis of variance are presented in table 3. The mean square for haemacytometer counts within ejaculates is significantly greater (probability <0.01) than that for Photelometer counts within ejaculates. These data indicate that the Photelometer gives more reproducible readings than does the haemacytometer for any individual semen sample. The mean square for the interaction between methods and ejaculates is significantly greater than the average mean square for the within-ejaculate Photelometer and haemacytometer readings. This fact indicates that the main source of error in the comparison of these two methods of determining spermatozoan numbers is due to characteristics, inherent in the seminal plasma or the spermatozoa, which cause variation in light transmission readings between samples independent of spermatozoan numbers.

Summary

1. Five different procedures for determining spermatozoan numbers by measurement of light transmission with a Cenco-Sheard-Sanford Photelometer were compared. It was concluded that the most accurate and practical method consisted of the addition of 4 cc. of sodium citrate buffer to 0.1 cc. of semen and the making of the readings with the diluted semen in a standardized tubular absorption cell 17 mm. in diameter.

2. Haemacytometer counts and light transmission readings using this method were made with 142 samples from 50 bulls in 3 studs. A correlation of —0.97 between logarithms of Photelometer readings and haemacytometer counts was obtained. The regression coefficient was —0.28. There were no significant differences between the within-bull correlation or regression coefficients.

3. A comparison of readings made on nine Photelometers with five semen samples in duplicate indicated that an instrument would not need to be calibrated if it were to be used in a bull stud but that it should be if maximum accuracy, such as that required in some research work, is desired.

4. Different sources of variation when comparing haemacytometer counts with counts estimated by means of light transmission values were determined. Duplicate readings by both methods were made with 25 ejaculates from 20 bulls. It was found that the Photelometer will give more reproducible readings for any given semen sample than will the haemacytometer. The main source of error when comparing the two methods is variation in the inherent properties of the seminal plasma or spermatozoa which cause variation in light transmission readings independent of spermatozoan numbers.

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THE TECHNIQUE FOR CHARACTERIZING MAMMALIAN SPERMATOZOA AS DEAD OR LIVING BY DIFFERENTIAL STAINING

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THE technique employed by numerous investigators for making differential counts between living and dead cells is that in which the so-called vital stains are used. Bichert (1930), Gay and Clark (1934), Knaysi (1935), and others have utilized this technique to differentiate living from dead bacteria. Knaysi has discussed the advantages and disadvantages of this method in bacterial investigations. In physiological studies of mammalian spermatozoa Lasley, Easley, and McKenzie (1942), Easley, Mayer, and Bogart (1942), Lasley and Bogart (1943), Lasley and Mayer (1944), Mayer and Lasley (1945), and Mayer, Squiers, and Bogart (1947), have made use of a vital staining technique. Schrek (1943a, 1943b, 1944, 1945) studied cellular suspensions derived from thymus, bone marrow and cancer tissues and based the results upon differential counts of stained and unstained cells in an eosin-containing medium. Later Schrek (1949) used a dilute solution of safranine in Tyrode's solution in bone marrow studies.

The staining mixture described by Lasley, *et al.* (1942) was composed of eosin and opal blue in isotonic phosphate buffer and was the first vital stain used for the differentiation of spermatozoa on the basis of their staining characteristics. The application of this original staining technique in numerous investigations in this laboratory indicated the necessity of further investigations of the staining principle, eosin. Opal blue (Breslau formula), the background stain, is a German preparation and has been unobtainable since the beginning of World War II. It is the purpose of the present publication to: (1) report investigations of the staining principle and factors affecting the differential staining of spermatozoa, (2) describe a new background stain to replace opal blue, and (3) examine the technique as a criterion whereby the living (non-stained) and dead (stained) spermatozoa may be differentiated.

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Materials and Methods

The present investigation was divided into two distinct phases: one in which the staining principle and factors affecting its optimal functioning were investigated; the other concerned with investigations of the background stain alone and in combination with the staining principle.

The former could be conducted most efficiently on the semen of a single mammalian species, the ram. Ram semen was selected because it possesses a relatively high spermatozoan concentration, it can be obtained without difficulty, and knowledge pertaining to its chemistry, metabolic activities and physiology is available.

The ram semen was collected with an artificial vagina into a clean sterile vial. After stoppering with a paraffined cork, the vial was placed in a thermos bottle containing water at 15° C. and kept at this temperature during the 15-to-20 minute period between collection and the experimental utilization of the semen. At 15° C. the metabolic rate of ram spermatozoa is reduced to less than one-twentieth of that at 37° C. as shown by Moore and Mayer (1941).

The second phase of this investigation was comprised of experiments on the background stain and the applicability of the differential staining technique to the spermatozoa of several species including the ram, stallion, boar, bull, rabbit and man. The human semen was obtained from donors of known fertility. All other semen specimens were collected with an appropriately designed artificial vagina.

The numerous stains investigated under various experimental conditions will be discussed in the description of the individual experiments in which each was used. Each stain studied, whether as a cellular staining principle or as a background stain, was always dissolved (unless otherwise indicated) in an M/8 phosphate buffer which is approximately isotonic for mammalian spermatozoa and has a pH of 7.30 to 7.4.

All solutions, including the phosphate buffer, were prepared with twice distilled water; the second distillation being made in an all glass distilling apparatus.

The slides upon which the spermatozoa could be separated into unstained cells and cells stained a pink to a deep red against a background of a contrasting color were prepared as follows:

1. Place one large drop[3] of stain[4] about one inch from the end of a very clean slide.

[3] Knowledge of just how much stain to use may be acquired, in part, from experience.
[4] If the stain has been kept in the refrigerator, it should be warmed to room temperature before using.

2. Dip a clean glass stirring rod[5] into the semen sample and then mix the adhering semen well but quickly with the stain droplet.

3. Place a second clean slide directly on the first, leaving enough space uncovered by stain on each slide to allow space for marking.

4. Immediately apply a soft cloth or towel to the edges of the two slides, applying slight pressure and at the same time drawing off excess stain with the towel.

5. Separate the two slides quickly, using a sliding action, and hold immediately under the hot air blast from a hair dryer until dry.

Slides should be prepared and dried within a few seconds after the staining procedure is started. Unsatisfactory results may be attributable either to the use of improperly cleaned slides or to delays during the process of making slides.

Experimental and Results

The Staining Principle

The original staining mixture containing eosin B and opal blue[6] (Lagerlöf, 1934) in an isosmotic phosphate buffer (pH 7.35) had been prepared for an investigation of the so-called "protoplasmic droplets" found under prescribed conditions upon mammalian spermatozoa. When the property of this staining mixture to separate spermatozoa in a specimen into a group of stained or dead cells and a group of unstained and presumably live cells was discovered, the technique was published prior to any thorough investigation of the optimum conditions or the mechanism of this differential staining method. The experiments reported are the results of an endeavor to ascertain the optimal chemical and physical conditions necessary for accurate differentiation of spermatozoa into these two groups. In addition, it was thought that the results obtained might give some indication of the mechanism involved.

In the following exposition of the results of numerous experiments endeavoring to identify stains specific for assessing the viability of mammalian spermatozoa and to establish the optimum conditions for the staining technique, it will be impracticable to report detailed experimental procedures and results. Instead a summary of the results of pertinent experiments, substantiated by numerous repetitions under controlled conditions, will be presented.

Stain specificity. Numerous so-called vital stains have been used in the differentiation of live from dead cells in studies of yeasts, bacteria, and plant tissues; eosin, however, so far as the authors are aware, is

[5] Amount of semen used may be regulated by size of stirring rod. Proportion of semen to stain should be conditioned by spermatozoan concentration.

[6] Opal Blue Solution according to Breslau, Dr. G. Grübler and Co., Eimer and Amend, New York.

the only substance used to obtain this type of differentiation with mammalian spermatozoa and cells of other mammalian tissues with the exception of Revector soluble blue counterstained with neutral red used by Crooke and Mandl (1947), for assessing the viability of human spermatozoa.

In the initial experiments using ram spermatozoa as the test cell, no background stain was used in order that the type of dyes capable of differentially staining these cells and the optimal conditions under which differential staining would occur could be more thoroughly studied.

A solution containing 0.4 percent eosin B in a buffer (pH 7.35) comprised of 1 part M/8 monosodium phosphate and 4 parts of M/8 disodium phosphate was found to give excellent differentiation in the absence of a background stain and slides made with this staining mixture were used as controls in the various experiments on the staining principle. A green or blue filter placed in the microscope was beneficial in these studies.

On the basis of the results of these preliminary experiments with eosin B, the stains to be studied were dissolved in the isosmotic M/8 molar sodium phosphate buffer at the 0.4 percent concentration. Some of the stains were insoluble and a few were soluble at concentrations below the 0.4 percent level.

Neutral red, acid fuchsin, congo red, carmine red, and Sudan III did not differentiate the cells into stained and unstained groups. Sudan III was insoluble in the buffer solution. Neutral red and carmine red stained none of the cells. Methyl red and acid fuchsin stained all of the spermatozoa faintly, even when eosin B was incorporated in the staining mixture. At concentrations of 0.1 and 0.2 percent the acid fuchsin stained the spermatozoa without disturbing the cellular structure, whereas at higher concentrations cellular disintegration occurred. Methylene blue and related dyes also proved ineffective.

Eosin B is a water-soluble halogen derivative of fluorescein, a member of the fluoran dye group. Since none of the stains mentioned in the preceding paragraph nor a number of others tested in experiments with the background stain were effective and eosin B had repeatedly given excellent results, a study of the water-soluble halogen derivatives of fluorescein was made. Eosin B, eosin Y, erythrosin B, and rose bengal were then used in the M/8 buffer mixture at different concentrations and under varying conditions.

In general, the results of a number of experiments over a period of a year using fresh and stored ram semen may be briefly summarized as follows:

1. All of the listed water-soluble halogen derivatives of fluorescein sharply differentiated a semen specimen into a stained and unstained group. However, eosin B and erythrosin B stain the spermatozoa a deeper red permitting the counting of stained and unstained cells with greater accuracy and facility.

2. Since all of the stains used differed in the number and kind of halogen substitution in the parent molecule, this difference in the halogen substitution groups apparently had little influence on the differential staining mechanism.

3. The optimal concentration of the fluorescein derivatives in the isosmotic phosphate buffer was 0.4 percent in the absence of a background stain.

Effect of pH. A series of phosphate buffer mixtures were prepared with pH values from 4.8 to 8.6; the interval between pH values of the mixtures was approximately 0.3 of a pH unit. Stain mixtures were prepared with each of the buffer mixtures and eosin B at the 0.4 percent concentration and tested on the same specimen of ram semen. Below a pH value of 5.8, agglutination of the spermatozoa occurred. All of the spermatozoa were stained when the pH of the staining mixture was below 6.4 or above 8.5. The best results were obtained with staining mixtures possessing pH values between 6.8 and 7.8. Above pH 8.5 the heads and tails of many of the spermatozoa were separated.

The eosin B or erythrosin B was dissolved in staining media containing a variety of substances instead of the phosphate buffer. In some cases isosmotic concentrations of these substances were added; while in others, especially in those cases where the exact concentration of an isotonic solution was not known, different concentrations were tried. The anion or the cation of the staining medium was changed by substituting appropriate salts for the phosphate buffer. Not only substances which did not ionize in solution but those which were highly ionized were also used in the preparation of the staining medium.

It was found that the staining mechanism apparently is dependent upon the presence of a substance which is ionizable in the staining medium. If the fluorescein derivative was dissolved in isosmotic glucose or sucrose solutions, none of the spermatozoa were stained.

However, a buffer salt is not a necessary constituent of the medium so long as the semen is not too acid, as a staining mixture of 0.4 percent eosin B or erythrosin in 0.9 percent NaCl with a pH of 7 gave excellent results.

Neither calcium chloride nor calcium lactate proved to be good substitutes for the phosphate buffer. The calcium chloride completely precipitated the stain from solution; whereas calcium lactate partially precipitated the stain. In both cases the dead cells were faintly stained permitting a differential count which checked with that made with control staining mixtures on the same semen specimen.

Three other sodium salts, sodium citrate, sodium tartrate and sodium succinate, added to the staining medium in isosmotic concentrations influenced the differential staining in different ways. The citrate staining medium differentiated the dead and live spermatozoa as well as the phosphate buffer medium. However, the sodium citrate formed large crystals on the slides which interfered with the counting of the two types of cells under the microscope. The succinate-containing stain mixture yielded excellent slides with sharp differentiation, but the number of dead cells was greater than those on the control slides and the number of dead cells increased as the concentration of the sodium succinate in the media was increased or decreased from a 2 percent level. Apparently, this salt in the concentrations used, 0.5 percent to 8 percent, is toxic to ram spermatozoa.

The amino acid, glycine (NH_3free) at a 1 percent concentration in the staining medium afforded a faint but distinct differentiation, the percentage of stained and unstained cells being the same as that obtained with the erythrosin-phosphate buffer staining mixture. The spermatozoa were faintly stained with a diffuse area of stained material around them. The unstained cells had an indistinct outline and were difficult to observe.

The Background Stain

Since the original background stain, opal blue, proposed by Lasley, *et al.* (1942) was no longer available, a search for a new background stain was initiated. The background stain must impart to the intercellular spaces a color against which the unstained and the stained spermatozoa are distinguishable. In addition, the background stain should be a substance which is readily soluble in the staining medium, incapable of staining either the dead or viable spermatozoa, but has an affinity for the intercellular semen plasma residue on the slides. After thoroughly investigating a number of stains with these characteristics; fast green FCF, a soluble diaminotriphenylmethane derivative, proved to be superior under all the various experimental conditions. This stain, when used in the proper concentrations, gives a green homogeneous background which is restful to the eyes and which brings both stained and unstained cells into sharp relief. On a slide stained with a mixture of fast green and eosin B, dead spermatozoa are a deep purplish red; while the live or viable spermatozoa are unstained, although underlying green background tends to give them a slight greenish cast.

Trials using ram, boar, stallion, bull, rabbit and human semen were made in an endeavor to find the optimum concentrations both of eosin and of fast green in the phosphate buffer staining medium which would give the most usable stain from the standpoints of accuracy of differentiation, ease of counting, and degree of contrast between live and dead cells. The combination which gave the best results with the semen of all species investigated, except the boar, was prepared as follows:

TABLE 1. A COMPARISON OF FAST GREEN-EOSIN WITH OPAL BLUE-EOSIN STAINS IN THE DETERMINATION OF PERCENT OF DEAD SPERMATOZOA

Species	No. of samples	No. of sperm counted	Average % Dead with Opal blue-eosin	Average % Dead with Fast Green-eosin
Man	5	3,000	22.8	23.0
Bull	10	7,800	59.1	59.9
Ram	6	3,200	45.3	44.0
Rabbit	13	8,000	40.1	40.4
Stallion	5	3,500	57.9	58.2
Boar	11	7,600	35.2	37.6
Total	50	33,700		
Averages			44.21	44.92

Chi Square=0.9588 (based on the total).
Chi Square value required to show significant difference=3.841.

Fast green, FCF; 2 grams
Eosin B (bluish); 0.8 grams
M/8 phosphate buffer (pH 7.3–7.4); 100 ml
The staining mixture has a pH of approximately 7.25.
Erythrosin (bluish) or rose bengal may be substituted for eosin B in the staining mixture.

Our experience indicates that none of the live-dead staining methods reported to date gives consistently satisfactory results with boar semen. Apparently, one of the semen plasma constituents interferes with the differential staining. Boar epididymal spermatozoa do not present similar difficulties.

In table 1 are presented comparable data obtained with the eosin-fast green and eosin-opal blue staining mixtures using the semen of six species.

Discussion

Biologists have proposed many criteria whereby a living cell may be distinguished from a dead cell. Few of these criteria can become the

basis of a simple method for the differentiation of living and dead cells. Two of the most universal and important attributes of living cells, respiration and selective permeability, have formed the basis of vital staining techniques in which the oxidation-reduction potentialities of the living cell caused a characteristic color change in the dye or selective permeability determined whether the living cell was stained by a particular dye.

In the technique for the differentiation of mammalian spermatozoa into dead and viable groups on the basis of their staining characteristics, the exact mechanism is unknown. If intracellular substances are stained in the dead spermatozoa, then selective permeability may be involved. Thomas and Mayer (1949) working with boar spermatozoa isolated an acidic protein which precipitated at pH 6.0. When this protein was completely removed from the spermatozoa, they were unstainable with eosin. This indicates that intracellular staining and selective permeability as factors might be involved in the differential staining technique.

However, the lack of any staining by eosin in non-ionized sugar solutions and the failure to obtain differential staining except within a narrow pH range as reported in this investigation suggests that other factors may be involved. Perhaps, the staining of spermatozoa by eosin may be a surface phenomenon dependent upon the nature of the charge at the cell surface.

Under certain conditions, three types of spermatozoa are found on the slides stained by the differential staining technique: unstained cells, stained cells, and partially stained cells. Only the tails and midpieces are stained in these partially stained cells suggesting that the functioning of this part of the atypical mammalian male germ cell may be disturbed, while the head which carries the genes and is of utmost importance in fertility may be physiologically normal. Further investigations on the partially stained spermatozoa may show whether this phenomenon bears any relationship to the process of fertilization.

In physiological and biochemical research at the cellular level cell masses composed of millions of cells must be studied. The effects of changing environmental conditions by the addition of beneficial or of detrimental substances may be more accurately ascertained if the number of viable cells present at the beginning and at end of the experiment is known. Metabolic data obtained in respiration or glycolysis studies would be more reliable if calculated on the basis of viable cells rather than the total cell count. It is evident, then, that differential staining techniques can be a valuable aid in biological investigations

on all types of cells including the atypical cell, the mammalian spermatozoon.

Summary

1. A staining technique has been described which separates mammalian spermatozoa into two groups; viz., stained or dead cells and unstained or presumably live cells.
2. The differential staining technique has been shown to be applicable to human, ram, bull, rabbit, and stallion spermatozoa. A substance present in the semen plasma of the boar interferes with the differential staining of the spermatozoa of this species.
3. The water-soluble halogen derivatives of fluorescein were the only dyes studied capable of differentially staining mammalian spermatozoa.
4. A new background stain, fast green FCF has been investigated and found superior to opal blue for this purpose.
5. Studies with the staining principle have shown that differential staining is dependent upon the presence of ionizable substances and that the pH must be held within a narrow range for optimal staining results.

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DIGESTIBILITY COMPARISONS BETWEEN STEERS AND LAMBS FED PRAIRIE HAYS OF DIFFERENT QUALITY[1, 3]

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IT is agreed generally that the ability of ruminant animals to digest roughages and fibrous feeds far surpasses that of simple-stomached animals but information is far from complete on the comparative digestibility of various classes of feeds by different species of ruminants. Digestion coefficients of feeds from trials conducted with sheep often have been assumed to be equally applicable to cattle so far as these particular feeds are concerned, and coefficients from digestion trials with cattle have been applied to similar feeds for sheep. It is of value both in feeding and digestion studies to gain more information on what degree of accuracy or error may be expected in applying the results from trials with one species of ruminant to nutritional problems involving another species of ruminant. If sheep and cattle on the same feeds showed similar results in digestibility studies and feeding trials, it would be much simpler and less costly to employ sheep in further studies with such feeds.

If the results showed differences between the species it would be of value to know in what respects and to what extent the digestibility of the feeds by the two species differ so that designs in experimental work can be made accordingly.

Many of the early digestion studies involving comparisons between cattle and sheep were not extensive and too few animals were used to arrive at definite conclusions. Bartlett (1904), using one to three sheep and two steers, reported greater capacity in steers for digesting coarse, low-protein fodders. He also noted greater individual differences between sheep than between steers or between the two species. Hamilton and Kick (1927–28) fed two hays and reported that steers digested them better than sheep did. The difference between species was highly significant in the case of timothy hay.

Forbes *et al.* (1937) reported a series of comparisons between steers

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and sheep, using a larger number of animals than Hamilton and Kick used. Species differences were encountered, and it was suggested that separate digestion coefficients should be published for each species.

Briggs *et al.* (1947) used four lambs and eight steers in trials involving corn, dried sweet potatoes, and basal rations of: (1) cottonseed meal and prairie hay and (2) alfalfa hay. These workers reported some differences, though mostly of small magnitude. The greatest and probably the only significant difference reported was a difference in digestibility of protein from sweet potatoes, the average coefficient for steers being 50.8 percent and for lambs 19.8 percent.

Watson *et al.* (1948) used a sufficiently large number of animals (minimum of four) for comparisons between steers and sheep. Some differences were reported, but these workers concluded that most differences between species with the feeds tested were not great.

Materials and Methods

Two series of digestion trials were conducted to study this problem. In the first series nine ten-month-old lambs and nine nine-months-old steers were used. A 3 x 3 latin square design was used and prairie hay cut at three stages of maturity, i.e. early, medium, and late, was fed. Each animal received each type of hay during the trial. In the second series of trials four types of prairie hay were fed, namely, hay cut at early, medium, and late stages and early-cut hay that had been stored two years. Eight lambs and eight steers of about the same age as the animals used in the first trial, were used in this trial though no animal received all of the types of hay during the trial. Each hay was tested on six lambs and six steers. All lambs were fed the same amount and all steers were fed the same amount during each of these trials. In the first trial the collection and preliminary period were each of ten days duration, whereas in the second series of trials the preliminary period was ten days in length and the collection period was seven.

The feces from both the lambs and steers were collected by means of canvas sacks fastened to the animals by canvas harnesses similar to those used by Forbes (1937). The daily feces samples were preserved with thymol in tightly covered jars and stored at 0° Centigrade. Proximate chemical analyses (A.O.A.C. 1945) were made at the close of each trial on composite aliquoted samples of feed offered, refused feeds, and feces.

The coefficients of digestibility were determined in the manner explained by Maynard (1947).

The method of analysis of variance described by Snedecor (1946) was used to determine differences between the apparent digestion coefficients of the two species.

Results

The average coefficients of digestibility of hay cut at various stages of maturity are given in table 1 for the first series of trials. The

TABLE 1. TRIAL SERIES NO. I. AVERAGE AND STANDARD ERROR OF APPARENT DIGESTION COEFFICIENTS FOR STEERS AND LAMBS FED HAYS CUT AT THREE STAGES OF MATURITY

Hay Maturity and Test Animal	Dry Matter %	Protein %	Ether Extract %	Crude Fiber %	Nitrogen-Free Extract %
Early					
Steers					
Av.	47.61	43.96	50.81	55.20	50.88
St. error	.71	1.48	4.24	.81	.67
Lambs					
Av.	47.86	51.38	31.69	58.27	52.81
St. error	2.39	3.98	7.32	3.06	5.82
Medium					
Steers					
Av.	44.25	31.31	36.55	53.00	48.18
St. error	.94	2.08	2.46	1.43	.95
Lambs					
Av.	41.82	32.48	30.18	50.16	47.18
St. error	3.19	3.44	3.39	3.49	2.21
Late					
Steers					
Av.	38.38	6.70	32.09	48.88	42.56
St. error	.44	2.24	3.51	1.48	1.36
Lambs					
Av.	41.27	23.04	23.27	31.99	44.41
St. error	1.57	2.45	2.78	1.94	1.47

standard error of the coefficients for the two species of animals is shown.

In analyzing the results from the individual animals of each species it was apparent that there was considerable variation between animals of the same species receiving the same ration. This variation was greatest in lambs. This agrees with the work of Bartlett (1904).

In the first trial the difference between the average apparent digestibility coefficients for dry matter by the two species fed early cut hay was

only 0.25, for protein 7.42, for ether extract 19.12, for fiber 3.07, and for nitrogen-free extract 1.93. Ether extract is a minor component of fibrous feeds and digestion coefficients for this nutrient are usually more variable and unpredictable than for other nutrients. With the exception of ether extract, the average coefficients of digestibility were higher for lambs than for steers in the first series of trials.

The differences between the two species were not as great as those mentioned above when medium hay was fed. The difference between the averages of the apparent coefficients of digestibility was 2.43 for dry matter, 1.17 for protein, 6.37 for ether extract, 2.84 for fiber, and 1.00 for nitrogen-free extract. With the exception of protein, the steers had higher average coefficients than the lambs for each nutrient when medium cut hay was fed.

When late hay was fed the differences between the two species were considerably greater than was evident when early or medium cut hays were fed except in the case of ether extract. Further, there was no indication that one species digested the nutrients in the hay better than the other, since the difference between the two species in the average digestion coefficients of dry matter, protein, and nitrogen-free extract was 2.99, 16.34, and 1.85 respectively with the lamb having the advantage in all three instances. However, the digestion coefficients of steers were higher for ether extract and fiber than those of the lambs. The difference was 8.72 and 16.89 respectively for the two nutrients. While the differences between the average coefficients of digestibility between the two species is great in some instances, none of the differences were found to be significant. This is due likely to the large variation between animals within the same species.

Results from the second series of trials in which the digestion efficiency of steers and lambs were compared are given in table 2. When early cut hay was fed the steers showed greater average digestion coefficients than did the lambs and the dissimilarity is as follows for the following nutrients: dry matter, 5.96; protein, 6.76; ether extract, 9.99; fiber, 8.92, and nitrogen-free extract, 4.89. When all hays were considered and the data analyzed statistically, dry matter was the only component showing a between-species difference that was statistically significant.

When medium hay was fed, the steers surpassed the lambs in their ability to digest dry matter by 2.51, ether extract 6.64, fiber 1.24, and nitrogen-free extract 4.30. The lambs surpassed the steers in their ability to digest protein by 0.55. When fed late hay the steers surpassed the lambs in digesting dry matter, ether extract, fiber, and nitrogen-

TABLE 2. TRIAL SERIES NO. II. AVERAGE AND STANDARD ERROR OF APPARENT DIGESTION COEFFICIENTS FOR STEERS AND LAMBS FED HAYS CUT AT THREE STAGES OF MATURITY AND OF STORAGE HAY

Hay Maturity and Test Animal	Dry Matter %	Protein %	Ether Extract %	Crude Fiber %	Nitrogen-Free Extract %
Early					
Steers					
Av.	46.51	41.14	29.11	62.95	47.99
St. error	.74	.61	2.96	.84	.88
Lambs					
Av.	40.55	34.38	19.12	54.03	43.10
St. error	2.77	1.94	4.24	2.56	3.35
Medium					
Steers					
Av.	42.76	29.80	21.98	56.62	46.66
St. error	.94	1.04	3.43	1.35	1.27
Lambs					
Av.	40.25	30.35	15.34	55.38	42.36
St. error	2.02	3.41	4.72	3.24	2.48
Late					
Steers					
Av.	40.51	11.88	37.39	56.98	43.79
St. error	1.85	2.50	6.78	1.81	1.38
Lambs					
Av.	37.90	12.56	34.81	55.28	39.92
St. error	1.01	4.27	6.97	2.01	1.73
Storage					
Steers					
Av.	46.44	45.98	34.60	59.26	47.04
St. error	.66	1.57	9.64	1.11	.26
Lambs					
Av.	44.29	40.86	46.77	52.76	47.00
St. error	1.03	6.98	4.68	1.59	2.24

free extract by a difference of 2.61, 2.58, 1.70, and 3.87 respectively. The average apparent digestion coefficients of protein for lambs fed late hay was 0.68 higher than for steers. None of these differences were statistically significant.

The average apparent coefficients of digestibility of dry matter, protein, fiber, and nitrogen-free extract for steers exceeded those for the

lambs by 2.15, 5.12, 6.50, and 0.04 respectively, when storage hay was fed. The average apparent coefficient of digestibility of ether extract for lambs was 12.17 higher than for steers.

When the results from the two trials are averaged for each species there is little difference between the average coefficients of apparent digestibility for the feeds fed in these trials (table 3).
However, if the averages of all results are taken the steers had slightly higher digestion coefficients for all analyzed constituents of the rations except protein for which the digestion coefficients for the lambs were slightly higher. The differences were not always in the same direction

TABLE 3. OVERALL AVERAGES OF APPARENT DIGESTION COEFFICIENTS FOR STEERS AND LAMBS FED HAYS CUT AT THREE STAGES OF MATURITY

(Average from Both Series of Trials)

	Dry Matter	Protein	Ether Extract	Crude Fiber	N-Free Extract
	%	%	%	%	%
Steers	43.78	30.11	34.65	56.13	46.73
Lambs	41.99	32.15	28.74	51.12	45.25

in the individual comparisons, and in most cases were not great, with the result that the only significant difference was for dry matter, which occurred in the second series of trials.

The lambs reflected differences in the feeds to about the same degree as did the steers and, therefore, could be used to determine differences with about the same degree of accuracy, provided greater numbers were used to compensate for the greater variation that occurs between lambs fed the same type of feed. Further, since the discrepancy between species fails to show a definite pattern and on the whole is not extremely great, the application of digestion coefficients for one species to the other species would not result in serious errors for such purposes as calculating total digestible nutrients of the hays tested and applying such calculations to many ordinary feeding problems. The difference found in the chemical composition of similar hays from different areas would probably result in greater error than would result from species differences in arriving at coefficients to use in calculating an ordinary farm ration. However, if small differences in the average coefficients of digestibility will affect the results of an experiment, it may be wise to

use separate coefficients for each species. Thus, it would appear that where rather small differences were expected in the digestibility of two feeds, lambs would not be as satisfactory test animals as steers, since the extreme variation between lambs would of necessity require greater differences due to the treatment before statistical analysis would show a significant difference.

It should be recognized that lambs can be used satisfactorily as test animals in many cases. The cost of the test animals, feed, and equipment, to say nothing of the extreme advantage in ease of conducting a digestion trial, is in favor of lambs. Some of the disadvantages due to individual variability between lambs can be overcome by using somewhat larger numbers than if steers were used. It is the opinion of the authors that the advantages of using lambs would outweigh the disadvantages in testing many feeds and applying the digestion coefficients to a considerable part of the practical feeding work.

Summary

Two series of trials were conducted comparing the relative digestive capacities of steers and lambs fed different qualities of prairie hay. These trials involved fifty-one separate trials with each species.

The only statistically significant difference encountered between species throughout these trials was in the case of the digestibility of dry matter in the second series of trials. Other differences and tendencies that were encountered did not reach statistical significance. This may have been because any true differences were too small to reach significance with the number of comparisons used except in the case of dry matter which consists of all the other nutrients except water.

The digestion coefficients secured from the trials with steers showed less variation than those from the lamb trials.

Results from these trials indicate that where minimum variation between digestion coefficients is desirable together with the use of a minimum number of animals, steers are the more satisfactory test animals. However, lambs reflect differences between different feeds to about the same degree as do steers and where somewhat larger differences are being measured or where more animals can be used, the lower cost and greater ease in experimenting with lambs make them very desirable test animals to use. Coefficients secured from tests with lambs where several animals are used would probably be accurate enough to apply to cattle in computing the ordinary farm ration.

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A COMPARISON OF THE RELATIVE ACCURACY BETWEEN SEVEN-DAY AND TEN-DAY COLLECTION PERIODS IN DIGESTION TRIALS[1]

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THE length of the collection period in digestion trials is a somewhat controversial subject. Lengthening the collection period increases the labor, time, and expense involved as well as limiting the number of trials that can be conducted and the rations that can be tested with available facilities during a given length of time. Using longer collection periods also lengthens the "risk" period wherein accident, animal sickness, or other upsetting circumstances are apt to affect results adversely and necessitate repeating the trial. Shortening the collection period, on the other hand, may also give rise to inaccuracies due to the inability to secure feces collections that are as truly representative of the digestibility picture in quantity and/or chemical composition as might possibly be obtained from the longer collection period. While the highest possible accuracy is a worthy goal, the factors of time, labor, expense, and other considerations set limits to the extent to which a trial may be carried out in order to secure a further fractional degree of accuracy. It appears that the problem should resolve itself into an attempt to secure a practical degree of accuracy, using a minimum length of collection period to attain the degree of accuracy needed for the problem at hand. Many factors exert an influence on the results of a digestion trial and each should receive proper consideration. However, in deciding on the best length for the collection period, it is possible that all trials need not employ the same length of period, and that the problems and purposes involved in any particular trial should have considerable bearing on determining the length of collection period that should be used.

Various lengths of collection and preliminary periods have been employed by different investigators studying problems in digestibility. Few comparisons of the relative accuracy of different lengths of trials could be found. Knight, Hepner, and Morton (1906) used seven- and

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eight-day preliminary periods and five-day collection periods with wethers. Such periods yielded comparable results during two separate experiments on similar feeds with the same animals.

Mumford *et al.* (1914) in digestion trials with steers used two-week preliminary periods and two-week collection periods followed by a two-week transitional period preceding the next preliminary.

Sotola (1927) used ten-day preliminary periods followed by ten-day collection periods using sheep in digestibility studies with alfalfa. These lengths of periods have been used considerably during recent years both for sheep and cattle.

Illinois (1927–28) reported using a ten-day collection period for sheep and a fourteen-day collection period for steers preceded by several weeks preliminary feeding during digestion trials. This same report mentioned that, when chromium oxide was mixed into the feed, the feces were practically free of the compound within a few days after feeding of the material was discontinued.

Schneider and Ellenberger (1927) made an interesting study of the effect of length of trial on apparent digestibility. These workers used at least a week preliminary period and tested rations near maintenance level and at higher levels of nutrition. They reported that the greatest error encountered in calculating digestion coefficients was the irregularity in the quantity of feces excreted. This irregularity was more pronounced at low nutritional levels. According to these findings, digestibility results from a nine-day lactation trial (high plane of nutrition) gave approximately the same accuracy as results from a twenty-four day maintenance trial. There is a possibility which was not mentioned by the authors, that the greater error found between planes of nutrition may be partially due to greater individual difference caused by the differences in ration ingredients. Fraps (1925) reported that there is an even chance, when using animals of the same species, for a 3 percent individual variation with such feeds as corn and wheat, and about 14 percent individual variation with low grade feeds.

Hamilton *et al.* (1928) found, in feeding chromium oxide and ferric oxide, that the feces were practically free of these substances within three to four days after these materials were withheld from the rations. This would indicate that three to five days are required after changing rations before the previous ration would be completely eliminated from the digestive tract.

Hamilton, Mitchell, and Kammlade (1928) stated that collection periods of five or six days are too short for accurate results, especially with steers.

Newlander and Jones (1932) in digestibility studies with dairy cows, used two-week collection periods and one-week (one trial) and two-week (one trial) adjustment periods between collections.

Experimental

The total collection method was used, and the chemical analysis was according to A.O.A.C. standards. The experimental animals were nine grade Hereford range calves averaging 456 pounds.

During the trials the steers were fed, twice daily, rations near a maintenance level. The steers were stanchioned two hours during each feeding period, then turned together in an inside concrete-floored exercise pen until the next feeding time. Salt and water were available in the exercise pen at all times.

The three rations being tested were prairie hays cut at different stages of maturity, supplemented with one pound each of oats and pelleted soybean oil meal. The oats and pelleted soybean oil meal were fed in two equal feedings (one-half pound of oats and one-half pound of pellets at each feed) being put in the feed bunks before the hay was fed so that all oats and pellets were consumed.

The feces were collected by means of harnesses and sacks similar to those used by Garrigus and Rusk (1939). Sacks were changed twice daily, and one-fortieth aliquots of the day's collection were taken once daily and stored in glass jars in a refrigerator. Thymol was used as a preservative.

Twenty-seven individual trials were conducted for comparison purposes. A ten-day preliminary was followed by the collection period. Double feces samples were taken during the first seven days of the collection period after which time single samples were continued for the three remaining days. Refused feed was removed from the stalls twice daily and stored in covered metal containers until the end of the collection period, at which time the orts were weighed, sampled, and preliminary moisture determined before sending them to Experiment Station Bio-Chemistry Department for analysis.[3] Since the steers were eating in a relatively uniform manner during each of the trials, separate weights and analyses were not made on the refused feed for the seven-day and the ten-day periods. The weight and analyses of the refused feed for the ten-day period was multiplied by 0.7 to compute the nutrients in the refused feed for the seven-day period.

[3] Analyses done by A. L. Moxon and R. Wilcox, Experiment Station Bio-Chemistry Department, South Dakota State College.

The coefficients of digestibility were determined in the manner explained by Maynard (1947) and Morrison (1948). To determine the amount of each nutrient that was consumed, the weight of the hay offered was multiplied by the percentage chemical composition of each nutrient. If any of the feed was refused, this was collected, weighed, and such weight multiplied by the percentage chemical composition for each nutrient in the refused feed. This amount was subtracted from the amount of the nutrient offered, the difference being the amount consumed. The values for excreted nutrients were obtained by multiplying the weight of the feces by the percentage composition of each nutrient. To obtain the amount of each nutrient apparently digested, the amount of each nutrient voided was subtracted from the amount of the nutrient consumed. Dividing the amount of a nutrient apparently digested by the amount of that nutrient consumed, the apparent digestibility was obtained which when multiplied by 100 is expressed as a percentage.

Results and Discussion

Findings similar to those reported by Schneider and Ellenberger (1927) seemed apparent in this experiment in that the quantity of feces collected from day to day varied considerably. Chemical composition of the samples for the two lengths of collection periods did not appear to differ greatly, though in a few cases the differences were slightly greater than the analyst would allow for duplicate analyses of the same sample material. The prairie hay used was a mixture of grass species and varied somewhat in composition. Also, significant differences in apparent digestibility were found between periods, thus it cannot be expected that the samples from the two different lengths of collection periods would agree as closely in chemical composition as duplicate analyses of the same sample.

The digestion coefficients are of prime importance in digestibility studies. Means and standard deviations of the coefficients for the seven- and ten-day samples appear in table 1. Loss in efficiency of the seven-day period compared to the ten-day period was calculated as follows: The coefficient of variation (Snedecor 1946) is calculated for the digestion coefficients of each class of nutrients for each of the samples representing the period lengths. The coefficient of variation of one method is subtracted (always one way) from the coefficient of variation of the other method, the remainder being the percentage loss in efficiency. In this experiment, the seven-day samples were expected to have the larger coefficients of variation so the ten-day coefficients were subtracted from

the seven-day coefficients. The resulting losses in efficiency between the two methods are as follows:

Dry Matter	−0.021% loss in efficiency.
Ether Extract	+1.051% loss in efficiency.
Crude protein	+0.236% loss in efficiency.
Crude fiber	+0.782% loss in efficiency.
N.F.E.	+6.834% loss in efficiency.

The negative 0.021 percent "loss in efficiency" for dry matter favors the seven-day collection period. Such a small difference is of doubtful significance, but shows that cases arise where the seven-day collection period gives a degree of accuracy as great or greater than the ten-day period. "Difference in efficiency" might be more accurate terminology than "loss in efficiency" in comparisons of this kind, since the great variations in daily feces weights makes it difficult to know just when to

TABLE 1. COMPARISON OF THE MEANS AND STANDARD DEVIATIONS OF DIGESTION COEFFICIENTS FROM 7-DAY AND 10-DAY COLLECTION PERIODS

	Dry Matter		Ether Extract		Crude Protein		Crude Fiber		N-Free Extract	
	7-Day	10-Day	7-Day	10-Day	7-Day	10-Day	7-Day	10-Day	7-Day	10-Day
Mean	48	48	41	41	60	61	51	50	53	53
Standard Deviation	4.33	4.32	4.37	3.88	4.24	4.13	6.12	5.68	6.97	3.37

end the collection in order to secure most accurate and representative results in digestibility studies. A short collection period may in some instances give the better average digestibility of a feed. If other error-producing factors could be eliminated, the use of the longer collection period should act in somewhat the same manner as using greater numbers when sampling a population.

A comparison of the averages and standard deviations of digestion coefficients from the seven- and ten-day collection periods for the three rations tested are presented in table 1. As would be expected after examining the computed "losses in efficiency", nitrogen-free extract had greatest standard deviation differences between the seven- and ten-day periods.

The use of the seven-day collection period rather than the ten-day period in calculating digestion coefficients for the various nutrients in these trials appeared to cause very little loss in accuracy except in the case of nitrogen-free extract. Nitrogen-free extract is arrived at by difference and contains an accumulation of whatever errors were made during the analyses for the other nutrients. For this reason it is difficult

to ascertain how much of the calculated loss in efficiency was due to the use of the seven-day period and how much may have been due to an accumulation of analyses errors which are reflected in the determination of the nitrogen-free extract. It might be worth while to compare different collection period lengths using a more direct method of determining nitrogen-free extract, especially the use of some method that comes nearer to measuring the nutrient components that nitrogen-free extract is theoretically a measure of, rather than including a large portion of lignin (Crampton and Maynard 1937, Anderson 1948).

Summary

Coefficients of apparent digestibility were calculated from data secured from seven- and ten-day collection periods during twenty-seven individual trials. Relative accuracy was compared between the two lengths of periods by using coefficients of apparent digestibility and calculating loss in efficiency between the coefficients from seven- and ten-day periods. Loss in efficiency for the coefficients of the nutrients studied were less than 2 percent in all cases except that of nitrogen-free extract which showed an efficiency loss of 6.83 percent. Because of the nature of the analyses in determining nitrogen-free extract, it is difficult to evaluate and interpret the significance of the decreased efficiency encountered here.

The degree of accuracy secured by the seven-day collection period may be sufficient for many studies of apparent digestibility. With a possible exception in the case of nitrogen-free extract the seven-day collection period gave coefficients comparable in accuracy to the ten-day collection period.

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COMPARATIVE EFFECT ON VITAMIN A METABOLISM IN SHEEP OF UREA, SOYBEAN OIL MEAL AND COTTONSEED MEAL AS SOURCES OF PROTEIN[1]

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A NUMBER of materials have been shown to influence the metabolism of carotene and vitamin A in domestic animals. Prominent among these are the tocopherols (Whiting, Loosli and Willman, 1949), lecithin (Eaton *et al.*, 1949) (Esh, Sutton, Hibbs and Krauss, 1948), and such metabolic stimulants and depressants as thyroxin, thiouracil and thiourea (Eveleth, Bolin and Goldsby, 1949) (Hibbs and Krauss, 1947) (Johnson and Baumann, 1948). Much of the contemporary literature dealing with the action of these compounds is reviewed in these references. That vitamin A itself has a marked effect on the absorption or metabolism of carotene is shown by the decreased carotene content of the blood plasma and milk of dairy cows following the addition of vitamin A to the ration (Deuel *et al.*, 1942) (Fountaine and Bolin, 1944). Recently an inverse relationship was found between the carotene and inorganic phosphorus content of the blood plasma of beef cattle on low- and adequate-phosphorus rations (Ross and Gallup, 1949). In view of the increased use of urea in feeds, it became desirable to know its effect on carotene and vitamin A utilization.

Procedure

As a general plan in these experiments, uniform groups of lambs were partially depleted in their stores of vitamin A by feeding a ration composed of cottonseed hulls, oats, high-protein oil meal and a mineral supplement for a period of about 3 months. Periodic determinations were made of the vitamin A content of the blood plasma, and at the end of the depletion period representative animals were slaughtered to get an approximate measure of liver storage of vitamin A. The remainder of the lambs were then divided into groups on the basis of body weight and plasma-vitamin A levels for experimental treatments.

During the experimental period which followed the depletion period,

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urea replaced an equivalent of about 50 percent of the nitrogen of the oil-meal supplement in the basal ration for half of the lambs, the other half being continued on the oil-meal rations. All lambs were given a supplement of carotene or vitamin A in amounts proportional to average body weight. The lambs were fed individually and weighed every two

TABLE 1. RATIONS FED SHEEP DURING EXPERIMENTAL PERIOD IN EXPERIMENTS 3, 4 AND 5[1]

Ingredients \ Ration identification		C.S.M.	C.S.M. plus urea	S.B.M.	S.B.M. plus urea
Experiment 3.					
Cottonseed hulls	percent	48.0	50.0	52.0	51.0
Oats	do.	30.0	38.5	30.0	39.5
Cottonseed meal	do.	22.0	10.0	—	—
Soybean oil meal	do.	—	—	18.0	8.0
Urea	do.	—	1.5	—	1.5
Protein equivalent	percent	13.2	13.6	13.0	13.6
Experiment 4.					
Cottonseed hulls	percent	0.3	3.0	1.6	3.7
Oats	do.	43.4	43.4	43.4	43.4
Cottonseed meal	do.	6.3	3.2	—	—
Soybean oil meal	do.	—	—	5.0	2.5
Urea	do.	—	0.4	—	0.4
Prairie hay	do.	50.0	50.0	50.0	50.0
Protein equivalent	percent	10.3	10.4	10.2	10.2
Experiment 5.					
Cottonseed hulls	percent	50.0	58.4	51.0	59.1
Oats	do.	30.0	30.0	30.0	30.0
Cottonseed meal	do.	20.0	10.0	—	—
Soybean oil meal	do.	—	—	19.0	9.5
Urea	do.	—	1.6	—	1.4
Protein equivalent	percent	12.4	12.6	12.5	12.5

[1] Vitamin A supplements used with these rations are shown in table 2. All animals had access to a mineral mixture of equal parts salt, ground limestone and bone meal.

weeks. Plasma vitamin A was determined at regular intervals, and vitamin A storage in the liver was determined at the end of the experimental period. Methods of analysis are given in a previous publication (Hoefer and Gallup, 1947).

In the first two experiments, alfalfa meal and a high-potency fish liver oil were used to supply carotene and vitamin A at two levels of intake representing minimum and ten times minimum requirements.

Since the details of these experiments have been reported in connection with the use of different vitamin A supplements for fattening lambs (Hoefer and Gallup, 1947) further comment is not warranted other than to point out that urea was without significant effect on the blood-plasma vitamin A values or the storage of vitamin A in the liver.

TABLE 2. VITAMIN A CONTENT OF PLASMA AND LIVER OF SHEEP FED DIFFERENT BASAL RATIONS AND DIFFERENT SOURCES AND AMOUNTS OF VITAMIN A

Ration identification	Number of sheep	Supplement feeding period days	Daily vitamin A intake [1]		Plasma vitamin A mcg. percent		Liver vitamin A mcg. per gram
			Source	Per kg. body weight i.u.	Initial	Final	Final
Experiment 3. Lambs. Preliminary depletion period. 115 days.							
C S. Meal	4	95	Fish liver oil	60	23	20	43
C.S.M.+urea	4	95	"	60	24	17	42
S. B. Meal	4	95	"	60	22	16	13
S.B.M.+urea	4	95	"	60	24	19	31
C S. Meal	4	95	Fish liver oil	500	23	26	186
C.S.M.+urea	4	95	"	500	24	23	228
S. B. Meal	4	95	"	500	24	27	183
S.B.M.+urea	4	95	"	500	24	20	207
Experiment 4. Lambs. Preliminary depletion period, 115 days.							
C. S. Meal	7	100	Prairie hay	400	22	25	32 [2]
C.S.M.+urea	7	100	"	400	18	22	
S. B. Meal	7	100	"	400	20	21	
S.B.M.+urea	7	100	"	400	20	22	
Experiment 5. Ewes. Preliminary depletion period, 30 days following winter feeding on low-carotene rations.							
C. S. Meal	6	60	Carotene conc.	550	28	33	164
C.S.M.+urea	6	60	"	550	28	39	136
S.B. Meal	6	60	"	550	24	35	94
S.B.M. +urea	6	60	"	550	22	37	101

[1] Based on 0.6 mcg. of carotene or 0.25 mcg. of vitamin A being equal to 1 I.U.
[2] Average of the four groups. Identification of the individual livers was not certain.

In the next experiment, designated as experiment 3 in tables 1 and 2, vitamin A was again supplied as fish liver oil at slightly over minimum and ten times minimum daily requirements. The oil had a potency of 40,000 I.U. per gram and was administered in capsules twice weekly. Minimum daily requirement was taken as 50 I.U. per kg. body weight. Two basal rations, one containing cottonseed meal and the other containing soybean oil meal, were compared with respect to their effect on vitamin A metabolism with two corresponding rations containing urea. The composition of these rations is given in table 1.

In experiment 4, prairie hay was used as the carotene source, replacing cottonseed hulls in the ration. The ration supplied approximately eight

times the daily requirements assuming 30 mcg. of carotene per kg. body weight per day as the minimum. During the later phases of the trial, it became necessary to replace a portion of the prairie hay with alfalfa leaf meal to maintain constancy of carotene intake. As in the previous experiment, urea replaced a portion of the nitrogen derived from either cottonseed or soybean oil meal.

Experiment 5 was conducted with a group of ewes which had previously been on winter rations of low vitamin A content. A carotene concentrate with a potency of 20,000 mcg. of carotene per gram was given as a source of vitamin A in amounts calculated to supply eleven times minimum daily requirements. The concentrate, diluted with corn oil, was added to the evening feed.

The composition of the basal rations is shown in table 1.

Results and Discussion

The lambs in experiment 3 at the end of the 115-day depletion period had an average plasma-vitamin A content of 24 mcg. per 100 ml. This value is about 10 mcg. lower than that usually found in comparable lambs fed fattening rations containing roughage of high carotene content. The livers from eight representative lambs slaughtered at the end of the depletion period contained an average of 62 mcg. of vitamin A per gm. During the next 95 days, when the daily ration was supplemented with fish liver oil in amounts to supply 60 I.U. of vitamin A per kg. body weight, blood values decreased somewhat; and, at the end of the period, the average liver value in one group was only 13 mcg. per gm. During the same supplement feeding period, plasma vitamin A in the lambs which received 500 I.U. of vitamin A was maintained at a level of about 24 mcg. per 100 ml. and liver stores were increased to about 200 mcg. per gram. Average values for the different groups of lambs on the different basal rations supplemented with these two amounts of vitamin A are shown in the first part of table 2.

In experiment 4, following a depletion period of 115 days, lambs which received vitamin A at the rate of 400 I.U. daily per kg. body weight in the form of carotene in prairie hay maintained plasma-vitamin A levels at about 22 mcg. per 100 ml. Livers from representative lambs slaughtered at the beginning of the supplement feeding period contained an average of 17 mcg. of vitamin A per gram; at the end of the period the average value was 32 mcg. From these results and earlier ones (Hoefer and Gallup, 1947) it is believed that an intake of approximately eight times minimum carotene requirements is necessary to secure appreciable storage of vitamin A in the liver of lambs.

Identity of individual livers from this group of lambs after slaughter was uncertain; consequently, data were not obtained as to the possible influence of the different rations on the liver values.

The ewes in experiment 5, despite prolonged feeding on low-carotene rations and a 30-day depletion period on a carotene-free ration, had slightly higher initial plasma-vitamin A values than the lambs used in experiments 3 and 4. During a period of 60 days in which they received 550 I.U. of vitamin A per kg. in the form of carotene their blood values increased from an initial value of about 26 mcg. to about 36 mcg. per 100 ml. Subsequent work has borne out this observation that ewes are able to maintain relatively higher plasma-vitamin A levels on limited vitamin A intakes than lambs. Average liver-vitamin A values, which ranged from 94 to 164 mcg. per gm., were much higher for the ewes than for lambs in experiment 4 which had received daily about two-thirds as much carotene, but they failed to reach the value for lambs in experiment 3 that had received 500 I.U. of vitamin A in fish liver oil. The greater efficiency of vitamin A as compared to carotene in promoting storage in the liver has been observed in earlier work.

The source of protein in the basal rations used in these experiments had little effect on the metabolism of vitamin A as measured by its content in the plasma and liver following supplemental feeding. Although in three of the four comparisons in experiment 3 the plasma of the lambs on the urea rations contained slightly less vitamin A than their controls, these differences were in the opposite direction in experiments 4 and 5 and were not found to have statistical significance. Liver vitamin A values were slightly higher for lambs which received urea in three of the four comparisons in experiment 3 but were lower or essentially the same as the controls in experiment 5.

In each of the six possible comparisons of cottonseed meal with soybean oil meal as the protein supplement in the basal rations, animals receiving the soybean oil meal rations had the lower liver vitamin A values. Because of wide individual variations within groups, however, these differences were not statistically significant. Thus, it seems that the observed influence of certain soybean products on absorption and utilization of vitamin A (Hauge *et al.*, 1940) (Squibb *et al.*, 1948) may be of no practical importance in the use of soybean oil meal in rations for fattening lambs.

Summary

Comparable groups of sheep which had been partially depleted in their stores of vitamin A were fed low-carotene rations containing soybean oil meal, cottonseed meal and urea as the principal sources of

nitrogen. In different experiments they were given supplements of high-potency codliver oil, prairie hay and a carotene concentrate as sources of vitamin A or carotene. Vitamin A was determined periodically in the blood. Its storage in the liver was determined at the beginning and end of the supplement feeding period.

The marked efficiency of fish liver oil as compared to carotene supplements in promoting storage of vitamin A in the liver was demonstrated. Although in each experiment there were differences in blood and liver-vitamin A values related to differences in the source of nitrogen in the basal ration, these differences were not of statistical significance. The trends of results indicated greater vitamin A storage when cottonseed meal was fed than when soybean oil meal was the protein supplement. Urea was without effect on carotene and vitamin A metabolism as measured by the plasma and liver values.

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LEVELS AND SOURCES OF PROTEIN IN RATIONS FOR PREGNANT EWES[1, 2]

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NUMEROUS publications have shown a great variety of protein to be of equal value when fed to sheep. Skinner and co-workers (1923, 1925) and Willman *et al.* (1946) compared the value of several protein supplements when fed to growing and fattening lambs. Miller and Morrison (1942, 1944); and Turk, Morrison and Maynard (1935) found no distinct differences in the utilization of protein from several sources when fed to growing lambs in metabolism experiments.

Harris and Mitchell (1941) and Johnson *et al.* (1942) reported that growing lambs are able to utilize urea but that this non-protein nitrogen is somewhat inferior to the nitrogen supplied by true protein. Loosli and Harris (1945) and Lofgreen *et al.* (1947) demonstrated that methionine supplementation markedly improves the utilization of urea nitrogen by growing lambs. Lofgreen and co-workers (1947) also reported that egg protein was utilized more efficiently than that supplied by linseed meal or urea plus methionine.

The information available on the amount of protein required by pregnant ewes is much more limited. Jordan *et al.* (1949 and 1950) fed rations varying from 3.5 percent to 10.5 percent digestible protein to ewes during gestation. No significant differences were found between these rations in regard to lamb or wool production even though the gains of the ewes during the pregnancy period varied considerably. The results of one experiment, published as a progress report by Van Horn and co-workers (1950), showed no marked differences in production from ewes fed pellets which varied from 10.4 to 37.0 percent crude protein as supplements to winter range.

The experiments presented in this paper were designed to determine the effect of feeding different levels of protein obtained by including various amounts of linseed meal or dried skimmilk as a protein supple-

[1] Contribution from the Division of Animal Industry, Agricultural Experiment Station of the North Dakota Agricultural College, Fargo, North Dakota. Published with the permission of the Director of the Station.

[2] A progress report on Purnell Project No. 137, "A study of the effect of kind and amount of protein in the ration on the pregnant ewe and her lamb". Further experiments are in progress.

ment to a grass hay, shelled corn ration when fed to ewes during the flushing, breeding and pregnancy period. One of the objectives was to compare the number of lambs produced from ewes fed a low protein ration to those fed a liberal amount of high quality protein during the flushing and breeding.

TABLE 1. RATIONS FED, GAIN OF EWES AND LAMBS PRODUCED
(Experiment I, 1947–1948)

Lot No	I	II	III	IV
Total protein in ration, %	10.80	7.71	6.81	10.97
Dig. prot., lb./ewe/da.	0.33	0.21	0.18	0.33
T.D.N., lbs./ewe/da.	2.45	2.46	2.47	2.48
Average daily ration, lbs.				
Grass hay	2.43	2.43	2.43	2.43
Yellow corn	0.75	1.31	1.48	0.79
Dried skimmilk	0.75	0.21	0.05	—
Linseed Meal	—	—	—	0.79
Bonemeal	0.03	0.03	0.03	0.03
Salt[1]	0.03	0.03	0.03	0.03
No. of ewes per lot	10	10	10	10
Av. initial wt., lbs.	141.8	142.1	141.7	141.9
Av. gain during period, lbs.	40.2	36.0	31.1	31.0
Lamb production				
No. live lambs born	16	11	12	13
No. lambs born dead	0	1	0	0
No. ewes not lambing	1	2	2	2
Av. birth wt., single, lbs.	13.1	12.2	11.9	11.9
Av. birth wt., twins, lbs.	9.7	9.5	11.0	10.1
Av. lamb gain 1st wk.				
Singles, lbs.	6.9	4.7	5.9	5.7
Twins, lbs.	3.9	4.1	3.7	3.8
No. lambs died 1st week	1	2	1	0
Av. pounds lamb at one week per ewe lambed	24.0	17.5	22.1	24.0

[1] The salt used in all experiments was a commercial trace mineral salt which contained: Manganese, 0.455%; Iodine, 0.019%; Cobalt, 0.013%; Iron, 0.48%; Copper, 0.048%; and salt, 96.0%.

Procedure and Results

Experiment I

Forty grade Hampshire ewes were divided into four lots and started November 24, 1947 on their experimental rations. After they had received these rations for three weeks the ewes in all lots were bred to the same purebred Hampshire ram by moving him from one lot to

another at frequent intervals. The ewes remained on these rations until their lambs were one week of age at which time the experiment was terminated. The rations fed, gains made by ewes prior to lambing and lambs produced are presented in table 1.

TABLE 2. RATIONS FED, GAIN OF EWES AND LAMBS PRODUCED

(Experiment II, 1948–1949)

Lot No.	I	II	III
Total protein in ration, %	10.27	6.96	10.61
Dig. prot., lb./ewe/da.	0.33	0.19	0.34
T.D.N., lbs./ewe/da.	2.58	2.63	2.62
Average daily ration, lbs.			
Grass hay	3.03	3.07	3.02
Yellow corn	0.64	1.33	0.68
Dried skimmilk	0.64	—	—
Linseed meal	—	—	0.68
Bonemeal	0.02	0.02	0.02
Salt	0.02	0.02	0.02
No. ewes per lot	12	12	12
Av. initial wt., lbs.	129.3	130.7	130.3
Av. gain during period, lbs.	48.9	40.2	40.3
Lamb production			
No. live lambs born	17	15	14
No. lambs born dead	1	0	0
No. ewes not lambing	0	2	2
Av. birth wt., singles, lbs.	12.2	12.9	12.6
Av. birth wt., twins, lbs.	9.3	8.3	9.6
Av. lamb gain 1st week			
Singles, lbs.	5.1	5.4	4.8
Twins, lbs.	3.4	2.9	3.6
No. lambs died 1st week	1	0	0
Av. pounds lamb at one week per ewe lambed	19.2	20.4	21.1

Experiment II

Thirty-six of the ewes that had been used in the first experiment were divided into three lots. The procedure for this experiment was the same as in the previous trial except a purebred Columbia ram was used rather than a Hampshire. The ewes were started on the experimental rations on October 13, 1948. The results of this experiment are presented in table 2.

Experiment III

Ninety grade ewes of varied breeding were used in this experiment. The grade Hampshires used in the two previous experiments were included in this trial along with a group of Western ewes, some showing predominately Rambouillet breeding and others that were a cross from Western ewes and Shropshire rams. The experimental lots were equalized as nearly as possible with ewes of similar type.

In order to study with a larger number of ewes the effect of various rations fed during the flushing and breeding season upon number of lambs produced the ninety ewes were first divided into three lots. One lot was fed a liberal amount of dried skimmilk (the same ration as Lot I, table 3) a second lot received a medium level of protein with linseed meal as the protein supplement (Lot III, table 3) and the third lot was fed grass hay (Lot V, table 3). They were fed these rations three weeks prior to breeding and for the four weeks they were with the rams. Three purebred Columbia rams were used and were rotated between pens at twelve hour intervals. The results of this study showed no advantage in feeding a liberal amount of high quality protein during the flushing and breeding period. The 30 ewes fed dried skimmilk gave birth to 33 lambs, those fed corn and linseed meal 41 lambs and those fed hay alone produced 34 lambs.

After the ewes were bred six ewes from each of the three lots were started on each of the rations fed during the pregnancy period. The results of feeding five different rations during pregnancy are presented in table 3. The gains of the ewes presented in this table are for a period seven weeks shorter (flushing and breeding period) than those in Experiments I and II. In this experiment all ewes were fed the same ration after lambing so that any differences in lamb gains would be attributable to the rations fed during pregnancy. In this experiment the lambs were weighed when they averaged 60 days of age as well as at one week.

Experiment IV

In this experiment two groups of purebred Shropshire ewes were fed a low protein ration for approximately the last four months of pregnancy to determine whether or not the addition of DL methionine would have any beneficial effect. Miller (1944) and Stevenson *et al.* (1946) have shown that methionine has a nitrogen sparing action in the dog and rat, respectively.

TABLE 3. RATIONS FED, GAIN OF EWES AND LAMBS PRODUCED

(Experiment III, 1949–1950)

Lot No.	I	II	III	IV	V
Total protein in ration, %	10.09	10.42	8.11	6.89	5.63
Dig. prot., lb./ewe/da.	0.31	0.32	0.23	0.18	0.12
T.D.N. lbs./ewe/da.	2.49	2.54	2.50	2.51	2.09
Average daily ration, lbs.					
Grass hay	3.00	3.00	3.00	3.00	3.85
Yellow corn	0.60	0.64	1.00	1.22	0.40[1]
Dried skimmilk	0.60	—	—	—	—
Linseed meal	—	0.64	0.22	—	—
Cerelose	—	—	—	—	0.40[1]
Bonemeal	0.02	0.02	0.02	0.02	0.02
Salt	0.02	0.02	0.02	0.02	0.02
No. ewes per lot	18	18	18	18	18
Av. initial wt., lbs.	135.5	134.1	135.9	135.2	135.3
Av. gain during period, lbs.	26.1	23.8	27.4	25.3	1.5
Lamb production					
No. live lambs born	18	19	20	18	24
No. lambs born dead	2	0	0	2	0
No. ewes not lambing	2	4	2	2	3
Av. birth wt., singles, lbs.	13.3	12.4	12.5	12.2	10.6
Av. birth wt., twins, lbs.	10.2	11.1	11.5	9.7	9.5
Av. birth wt., triplets, lbs.	—	7.3	—	—	—
Av. lamb gain 1st week					
Singles, lbs.	3.2	3.8	4.3	3.4	3.2
Twins, lbs.	3.2	3.7	3.7	2.3	2.0
Triplets, lbs.	—	3.6	—	—	—
No. lambs died 1st week	1	0	2	1	5
Av. pounds lamb at one week per ewe lambed	16.6	20.1	18.5	14.3	16.9
Av. pounds lamb at 60 days per ewe lambed	42.1	45.5	45.9	35.4	36.2

[1] Mixture of equal parts yellow corn and cerelose fed during last 21 days only.

Starting at approximately the second month of pregnancy four ewes from each lot were used in a double reversal nitrogen balance experiment to determine the effect of methionine supplementation upon the utilization of the protein in this ration. These 8 ewes were fed in metabolism cages for six weeks. The results of this nitrogen metabolism experiment showed no apparent beneficial effect from the addition of 0.1 percent DL methionine to a low protein ration. The ewes, however, remained in positive nitrogen balance on this ration and stored an average of 9.6 grams of protein daily. The average daily ration fed and

the results obtained are presented in table 4. The daily allowance of digestible protein was calculated by using the apparent digestibility coefficients for protein determined in the metabolism experiment.

Discussion

The results of three experiments are presented in which the values of various sources and levels of proteins in rations for pregnant ewes have been tested. The results of a fourth experiment are given to show the

TABLE 4. RATIONS FED, GAIN OF EWES AND LAMBS PRODUCED
(Experiment IV, Shropshire Ewes, 1940–1950)

Lot No.	I	II
	Control	Plus 0.1% Methionine
Total protein in ration, %	6.99	7.00
Dig. prot., lb./ewe/da.	0.10	0.10
T.D.N., lbs./ewe/da.	1.74	1.78
Average daily ration, lbs.		
Grass hay	2.03	2.10
Dried beet pulp	0.95	0.96
Yellow corn	0.10	0.10
Bonemeal	0.04	0.04
Salt	0.04	0.04
No. ewes per lot	10	10
Av. initial wt., lbs.	121.3	122.0
Av. gain during period, lbs.	27.6	28.1
Lamb production		
No. live lambs born	13	10
No. lambs born dead	1	0
No. ewes not lambing	1	3
Av. birth wt., single, lbs.	10.1	10.6
Av. birth wt., twins, lbs.	8.2	8.6

value of adding approximately 0.1 percent methionine to a low protein ration and to show the gains of the ewes and birth weight of lambs produced from ewes fed this ration. The results from these experiments were not analyzed statistically. Observation of the data clearly shows that any differences in lamb production would not be statistically significant. Some differences were noted in the gains of the ewes but these differences had no apparent effect on lamb production. Fleece weights of the ewes were recorded but are not presented because the differences between lots were not consistent and well over one-half of the annual

fleece was produced on rations other than those studied in these experiments.

The number of lambs born and the number of ewes not lambing are presented rather than an average lambing percentage. An attempt was made to allot the ewes on the basis of the number of lambs they had produced during previous years. It was found, however, that the number of lambs a given ewe had from one year to the next was highly variable and it is doubtful if the rations fed had any influence on the number of lambs born. Many of the ewes that did not lamb during one year had either a single or twin lambs during other years. For this reason the figure of average pounds of lamb produced per ewe is based on the number of ewes that lambed rather than the number of ewes in the lot at breeding time.

The pounds of lamb produced per ewe is presented because it is believed to be the most practical measure of the value of the rations fed. This figure is an overall measure of the birth weight of the lambs, the number of lambs living to one week of age and the gain of the lambs during the first week. It will be noted from tables 1 to 3 that there is some variation between lots in regard to this measure but that no ration was consistently superior to others.

The ewes in Lot V, table 3, were not fed the same amount of total digestible nutrients as those in the other lots. It will be noted that their lambs were lighter at birth and that the death rate of lambs was higher. Had these ewes given birth to the same number of lambs their total lamb production would undoubtedly have been smaller than the ewes in the other four lots.

In the first three experiments the ewes were bled at regular intervals for oxyhemoglobin, serum total protein, albumin and globulin determinations. The results of these analyses during the first two experiments have been published (Klosterman *et al.*, 1950). The analyses obtained in the third experiment agree with the published results showing a reduction in oxyhemoglobin and serum albumin with lower amounts of protein fed. These blood constituents showed a more marked decline when the ewes were fed only grass hay and minerals than when corn was included in the ration.

The results obtained in Experiment IV, table 4, are not presented as conclusive information relative to the protein requirements of pregnant ewes. It is realized that these results are from only one experiment with one breed of sheep and one type of ration. This experiment does show, however, that pure-bred Shropshire ewes averaging over 130 pounds in body weight remained in positive nitrogen balance, made

satisfactory gains and produced thrifty lambs when fed an average of 0.10 pound of digestible protein per ewe daily during the pregnancy period. The National Research Council (Pearson *et al.*, 1949) recommended for 130 pound ewes 0.20 pound digestible protein during the first 100 days of gestation and 0.23 pound daily during the last six weeks before lambing. Further work is needed before recommendations can be made regarding the minimum amount of protein required by pregnant ewes.

Summary

The results of four experiments are presented. There were no consistent differences in lamb production from ewes fed rations approximately equal in total digestible nutrients but which varied from 6.8 to 11.0 percent total protein. There were no apparent differences in production between ewes fed linseed meal or dried skimmilk as a protein supplement to a grass hay, shelled corn ration.

Pregnant Shropshire ewes averaging 135 pounds in body weight remained in positive nitrogen balance, made satisfactory gains and gave birth to thrifty lambs when fed an average of 0.10 pound digestible protein per ewe daily. No improvement in utilization of protein or production was noted from the addition of 0.10 percent DL methionine. Further work is needed relative to the protein requirements of pregnant ewes.

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THE LEVEL OF COBALT TOLERANCE IN YEARLING SHEEP

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IT has been amply demonstrated that the ruminant species require infinitesimal quantities of cobalt in the diet and it is now recognized that cobalt-deficient areas exist in many of the important livestock regions of the United States, as well as in many foreign countries. Since cobalt deficiency occurs so widely, the feeding of cobalt salts has been recommended for the prophylaxis and therapy of this deficiency disease in cattle and sheep. However, the liberal and unregulated use of cobalt may result in toxic reactions and for this reason the tolerance level becomes an item of real importance.

Although the cobalt tolerance level of cattle has been well studied, the information on the cobalt tolerated by sheep is insufficient to warrant any conclusions. Geyer, *et al.* (1945) have noted that polycythemic reactions did not attend the feeding of 50 mg. of cobalt daily in the bovine. Furthermore, Ely, *et al.* (1946, 1948) reported a wide variation in the individual tolerance of dairy cows to high doses of cobalt, although dairy calves fed in excess of 40 mg. of cobalt daily per 100 lb. of body weight showed toxic symptoms in the form of reduced feed intake. Similarly, Keener, *et al.* (1949) found that growing dairy animals could consume up to approximately 50 mg. of cobalt per 100 lbs. of body weight without detrimental effects.

The authors are aware of only one investigation with sheep. Josland (1937) drenched four healthy ewes with 5 mg. of cobalt per kilogram of body weight, and reported that one developed polycythemia, two showed mild anemia, and the other remained normal. In addition, two ewe lambs became anemic after receiving 5 mg. of cobalt, in sulfate form, for a period of 10 months. Thus, the reactions were far from uniform.

Experimental Procedure

This experiment was initiated in February, 1949, as a part of a study designed to determine the role of cobalt in ruminant nutrition. The

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animals employed were western yearling wethers which had been carefully selected for thriftiness and freedom from disease and drenched with copper sulfate-phenothiazine to free them of internal parasites. The length of the trial varied depending on the level of cobalt intake. The groups on the lower levels of cobalt intake were continued for a period of eight weeks; whereas, the groups on higher levels were terminated at the conclusion of five weeks of dosage. The sheep were individually fed twice daily a ration consisting of shelled yellow corn (0.05 ppm of Co), soybean oil meal (0.25 ppm of Co), and good quality mixed legume hay (0.15 ppm of Co). The total ration contained approximately 11 percent crude protein and consisted of equal parts of concentrates and roughage. Block salt and water were available ad libitum.

Cobalt as $CoCl_2 . 6H_2O$, was administered orally at levels of 10, 20, 40, 60, 80, 160, 200, and 500 mg. of cobalt per centiweight per day in groups I through VIII, respectively. Precautions were taken to assure that the sheep received the entire dosage by dissolving the dose in a minimum amount of water. The dosage rate was computed for each week on the basis of the bodyweight of each lamb at the previous weigh day; body weights being recorded at weekly intervals.

Blood samples were obtained each week from the jugular vein and hemoglobin determined by the method of Sheard and Sanford (1929). Blood sugar values were determined during the sixth week of the study by the method of Somogyi (1930, 1937).

Results

The data in table 1 show the effect of various levels of cobalt intake upon the appetite of the sheep. The concentrate consumption was excellent for all groups, except groups VII and VIII which received 200 and 500 mg. of cobalt per centiweight daily, respectively. The latter groups showed a depressed appetite within two days following the initiation of cobalt administration, and the decrease continued until the sixth week when a moderate improvement was noted. This anorexia was the first noticeable symptom of cobalt toxicity in the sheep. Hay consumption followed a trend very similar to grain consumption in all groups.

With regard to the hemoglobin level of the blood (table 2), no differences were observed, except at the highest level of cobalt dosage. Sheep receiving 500 mg. of cobalt per centiweight per day showed a definite anemia, although the downward trend was accentuated by the death of three individuals in this group. Anemia was not observed in the

TABLE 1. AVERAGE DAILY CONCENTRATE CONSUMPTION OF YEARLING SHEEP ON VARIOUS LEVELS OF COBALT CHLORIDE

Group Number	1	2	3	4	5	6	7	8
Cobalt dosage, mg./cwt./day	10	20	40	60	80	160	200	500
Number of sheep	3	3	3	3	3	2[1]	5[2]	5[3]
Concentrates offered per lamb per day, lb.	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Concentrate consumption per lamb per day lb.								
Pretreatment Week	1.12	1.11	1.10	1.12	1.12	1.12	1.12	1.12
1st Week	1.12	1.12	1.11	1.12	1.12	1.12	.58	.36
2nd Week	1.10	1.11	1.10	1.11	1.11	1.11	.31	.21
3rd Week	1.12	1.12	1.10	1.12	1.12	1.12	.16	.04
4th Week	1.12	1.12	1.11	1.12	1.12	1.01	.04	.02
5th Week	1.12	1.12	1.10	1.12	1.12	1.12	.11	.01
6th Week	1.12	1.12	1.10	1.09	1.12	1.12	.71	.35
7th Week	1.12	1.11	1.10	1.11	1.12	1.12	—	—
8th Week	1.12	1.12	1.11	1.12	1.12	1.12	—	—

[1] One sheep removed after developing severe pulmonary inflammation.
[2] One sheep died during 6th week of test.
[3] Two sheep died during the 1st week, and one died in the 2nd week.

TABLE 2. A SUMMARY OF THE RESULTS OF FEEDING YEARLING SHEEP ON VARIOUS LEVELS OF COBALT CHLORIDE

Group Number	1	2	3	4	5	6	7	8
Cobalt dosage, mg./cwt./day	10	20	40	60	80	160	200	500
Number of sheep	3	3	3	3	3	2[1]	5[2]	5[3]
Average Body weight lbs.								
Initial	83.5	78.7	85.6	83.2	81.7	91.1	97.8	96.6
1st Week	84.9	81.2	87.6	82.8	83.9	93.8	92.3	87.8
2nd Week	84.4	81.3	85.8	83.0	81.1	91.4	88.2	78.4
3rd Week	86.5	83.8	89.0	85.3	81.3	92.6	85.3	73.4
4th Week	86.0	82.7	88.4	84.5	81.9	90.1	83.6	71.8
5th Week	88.4	85.6	87.9	84.6	83.1	91.3	85.0	64.6
6th Week	91.2	87.2	91.6	85.9	83.9	92.6	—	—
7th Week	91.8	89.7	91.7	87.1	85.8	90.6	—	—
8th Week	94.7	91.3	94.8	86.3	89.0	93.4	—	—
Mean Hemoglobin Level, gm./100 ml.								
Initial	9.56	10.26	10.67	10.05	11.77	10.20	10.03	9.98
1st Week	9.97	11.19	10.49	10.15	11.73	10.42	9.57	8.45
2nd Week	10.98	11.90	11.21	10.76	12.31	11.58	9.65	7.34
3rd Week	9.75	11.24	10.26	10.33	11.80	10.08	9.47	7.14
4th Week	10.48	11.35	10.88	10.86	13.44	11.56	9.20	6.82
5th Week	10.39	11.40	10.46	11.25	13.32	10.97	10.98	6.94
6th Week	10.03	10.40	10.83	11.10	13.39	10.56	—	—
7th Week	10.15	11.95	11.17	10.82	13.46	10.98	—	—
8th Week	10.21	10.96	10.72	10.89	12.51	10.49	—	—
Mean Blood Sugar level, 6th week, gm./100 ml.	56.7	58.0	62.7	67.0	60.7	60.3	—	—
Mortality	0	0	0	0	0	0	1	3

[1] One sheep removed after developing severe pulmonary inflammation.
[2] One sheep died during 6th week of test.
[3] Two sheep died the 1st week, and one sheep died in the 2nd week.

remaining groups nor were there any signs of polycythemia. Blood sugar determinations made during the sixth week of the test revealed insignificant differences in the groups tested. This observation is interesting in view of the findings of Stewart and Holman (1944) that cobalt-deficient sheep show a reduced blood sugar level, and also the discovery of Smith, *et al.* (1943) that polycythemia in rats produced by cobalt administration was accompanied by an elevated blood sugar level.

Mortality occurred only at the high dosage levels. One death occurred in group VII and three sheep died in group VIII. In the fatal cases, the sheep showed a stilted, incoordinated gait, and were rather excitable. This clinical condition progressed to a prostration which was accompanied by rapid and labored breathing. This latter stage immediately preceded death.

Pathological studies of the four sheep which died of cobalt toxicity revealed a fatty liver and slight pulmonary edema and congestion in all cases. The case from group VII showed an enlarged thyroid gland and numerous ecchymotic areas on the right ventricle of the heart. The remainder of the cases which were from group VIII showed petechial to ecchymotic hemorrhages in the small intestine, and inflammation of the abomasal mucosa was observed in one animal of this group. Another sheep possessed a red and grayish mottling of the lumbar and biceps femoris muscles, as well as an abnormally white bone marrow.

An additional study was made in an attempt to induce polycythemia in sheep by the intravenous injection of large doses of cobalt salts. Six animals which had been used in the above test were given daily intravenous injections of 75 mg. of cobalt as $CoCl_2.6H_2O$ solution. This intravenous dosage had been observed by the senior author to produce symptoms of distress in yearling sheep similar to those noted in the young bovine by Ely, *et al.* (1946) when 50 to 60 mg. of cobalt were administered intravenously. During an eight day period the mean hemoglobin values varied from 10.87 gm. per 100 ml. of blood on the initial day to a level of 12.64 gm. per 100 ml. of blood on the seventh day. Although the daily fluctuation in hemoglobin level was greater than normal, the mean values fall within the normal range for sheep noted by Becker and Smith (1950). On the eight day two sheep died without showing any evidence of polycythemia.

Discussion

Data presented herein show that yearling sheep are highly tolerant to an excessive oral intake of cobalt. Thus the possibility of cobalt

toxicosis occurring under practical conditions is unlikely. Such was also the conclusion of Josland (1937). Fattening yearling sheep in this test withstood a daily dosage of 160 mg. of cobalt per centiweight daily without any apparent undesirable effects for a period of eight weeks. This represents a cobalt intake nearly 1600 times the requirement of such sheep. That is, if we assume the intake level of Filmer and Underwood (1937) of 0.1 mg. of cobalt per day does approximate the minimum requirement for maintaining health in growing lambs.

Furthermore, the evidence obtained in this study is in agreement with the observations of Josland (1937) and Geyer, *et al.* (1945) who noted that it was difficult to produce cobalt polycythemia in the mature ruminant. In this regard, it is noteworthy that Keener, *et al.* (1949) showed that an increased hemoglobin level, erythrocyte count, and packed cell volume were the first effects of excessive cobalt consumption in young dairy cattle. In other words, dairy calves show the same response to excessive cobalt administration as the laboratory rat. Perhaps there is a difference in the reaction of mature and immature ruminants to large doses of cobalt salts which may be caused by a difference in the extent of rumen development. Actually, in this test the yearling sheep, which supposedly have a rather mature rumen, responded with an anemia when drenched with 500 mg. of cobalt per centiweight daily. However, the anemia possibly could have been the result of inanition in view of the severely depressed appetite. At any rate, it is not believed that failure to observe polycythemia in this study was a result of inefficient absorption of the cobalt, since death occurred with little or no elevation of hemoglobin when 75 mg. of cobalt was injected intravenously daily.

Summary

Data have been secured to determine the level of dietary cobalt tolerated by fattening yearling sheep. Yearling wethers fed a ration of shelled yellow corn, soybean oil meal, and mixed legume hay have been drenched daily with cobalt chloride at levels varying from 10 to 500 mg. of elemental cobalt per centiweight daily for periods up to eight weeks. Results obtained show that levels up to 160 mg. of cobalt per centiweight daily can be tolerated for at least eight weeks without harmful effects. Dosages of 200 or 500 mg. of cobalt per centiweight daily caused a severely depressed appetite and body weight losses; an anemia was noted at the higher level. Polycythemia was absent, and blood sugar determinations revealed no significant departure from

normal. Endeavors to produce polycythemia in six sheep by daily intravenous injections of cobalt at the rate of 75 mg. of cobalt daily resulted in death of two of the animals; there was no elevation of the hemoglobin level. Pathological studies of the sheep which died of cobalt toxicosis showed fatty infiltration of the liver, and slight pulmonary edema and congestion in all cases. In addition, three animals which received the highest cobalt intake also showed petechial to ecchymotic hemorrhages in the small intestine.

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LAMB PRODUCTION OF NAVAJO EWES BRED TO COLUMBIA AND ROMNEY RAMS, AND NAVAJO CROSSBRED EWES BRED TO LINCOLN AND COTSWOLD RAMS[1]

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THE major source of income for the Navajo people who raise sheep is from lambs sold on the market. A number of animals are used for home consumption. Hence, emphasis should be placed on selecting ewes capable of reproducing and raising lambs at a high rate.

The adaptability and high fertility of the old-type Navajo sheep make them desirable base stock in developing improved breeds suited to the environment of the semi-arid Southwest. Environmental conditions in some areas of this region have either prevented the utilization of highly developed breeds or sharply curtailed their productivity. The Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, is attempting to overcome this problem by the formation of new breeds, combining the adaptability and high fertility of the Navajo sheep with the high-producing qualities of certain pure breeds.

The objective of this study was to compare the lamb production of some of the crossbred sheep, which have been developed at the Southwestern Range and Sheep Breeding Laboratory.

Methods and Materials

This investigation was limited to comparisons of lamb production from 721 matings of Navajo ewes with Romney and Columbia rams, and 974 matings of Navajo crossbred ewes with Cotswold and Lincoln rams. The crossbred ewes have an inheritance of ½ Navajo, ¼ Romney and ¼ Corriedale breeding. Breeding and lambing records for these groups were analyzed for 1947, 1948 and 1949. Thirty purebred rams

[1] The data for this study were collected at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, under authority of the Bankhead-Jones Act, by the Bureau of Animal Industry, U. S. Department of Agriculture, in cooperation with the Bureau of Indian Affairs, U. S. Department of Interior, and the New Mexico Agricultural Experiment Station.

[2] U. S. Department of the Interior and collaborating with the U. S. Department of Agriculture. The assistance of Dr. H. H. Stonaker, Colorado A & M College is gratefully acknowledged.

were used: 10 Columbia, 5 Romney, 10 Lincoln and 5 Cotswold. A number of rams were used more than one breeding season. During the 3-year period, there was a total of 44 breeding pens with an average of 39 ewes per pen.

Unfortunately, it was impossible to include a control group of straight Navajo breeding in the study. The ewes in this group varied too much in age from the Navajo and crossbred ewes in the crossbred breeding groups for a valid comparison of lamb production.

The ewes were herded on browse-grass range at this station from about the first of October until breeding time in December. They were fed alfalfa hay and bred in pens for 30 days. They were herded on the fall and winter range until about the first of May and then trailed to the summer range in the Zuni mountains. In 1947, the ewes lambed in corrals at laboratory headquarters. In 1948 and 1949, the lambs were born on the summer range. They were weaned the latter part of August each year, and weaning weights were taken at that time. The average weaning age for the lambs was about 120 days.

Most of the rainfall in this semi-arid area comes during the late summer months. During 1947, 1948 and 1949 the rainfall was 12.64, 12.63 and 10.74 inches, respectively, and from lambing time in May to weaning time in September it was 8.12, 6.28 and 5.99 inches.

Results and Discussion

A preliminary analysis of the data showed no significant difference between years for weaning weights of lambs. All the ewes in the various breeding groups were in the same flock during the 3-year period; thus management and feed conditions were the same.

Lamb production of Navajo ewes bred to Columbia and Romney rams, and of Navajo crossbred ewes bred to Lincoln and Cotswold rams is summarized in table 1. The percentage of ewes lambing, of those bred and still alive at lambing time, was used as a measure of fertility of the different breeding groups. The percentage of lambs born from ewes having mature foetuses born at full gestation was used as a measure of fecundity. Viability was measured by the percentage of live lambs of lambs born and the percentage of lambs weaned of live lambs born. In table 2, the significance of differences between the various breeding groups was measured by the Chi-square test, based on actual numbers in each group (Fisher, 1941). There was no attempt to evaluate separately the merits of the ewes or rams as individuals.

Table 1 shows that in the crosses involving Columbia and Romney rams, the percentages of ewes lambing were 88.3 and 90.2, respectively. These figures are similar to the average of 88.5 percent of ewes lambing

TABLE 1. PERCENTAGE OF LAMB CROP AND POUNDS OF LAMB PRODUCED IN THE CROSSBRED BREEDING GROUPS FOR 1947, 1948 AND 1949

Breeding group	No. ewes	Percentage ewes lambing of ewes bred	Percentage lambs born of ewes lambing	Percentage live lambs of lambs born	Percentage lambs weaned of live lambs born	Average weaning weight in pounds	Percentage lambs weaned of ewes bred	Pounds lamb weaned per ewe bred
Columbia x Navajo	384	88.28	138.94	96.82	84.87	64.00	100.78	64.50
Romney x Navajo	337	90.21	140.46	99.53	84.47	61.38	106.53	65.39
Lincoln x crossbred	591	68.70[1]	143.60	98.80	84.38	63.29	82.23	52.05
Cotswold x crossbred	383	64.75[1]	142.34	95.75	82.25	65.11	72.58	47.26
Total or Average	1695	76.52	141.40	97.87	84.12	63.36	89.09	56.44

[1] See text regarding low fertility of some of the rams used.

TABLE 2. MEAN DIFFERENCES BETWEEN BREEDING GROUPS FOR FACTORS AFFECTING LAMB PRODUCTION FOR 1947, 1948 AND 1949

Characteristic	Columbia cross minus Romney cross	Columbia cross minus Lincoln cross	Columbia cross minus Cotswold cross	Romney cross minus Lincoln cross	Romney cross minus Cotswold cross	Lincoln cross minus Cotswold cross
Percentage ewes lambing of ewes bred	−1.93	+19.58**[1]	+23.53**[1]	+21.51**[1]	+25.46**[1]	+3.95
Percentage lambs born of ewes lambing	−1.52	−4.66	−3.40	−3.14	−1.88	+1.26
Percentage live lambs of lambs born	−2.71**	−1.98*	+1.07	+0.73	+3.78**	+3.05**
Percentage lambs weaned of live lambs born	+0.40	+0.49	+2.92	+0.09	+2.22	+2.13

* denotes significance at the .05 level.
** denotes significance at the .01 level.
[1] see text regarding low fertility of some of the rams used.

found by Blunn (1943), in 1,839 matings of Navajo rams with Navajo ewes. The percentages of crossbred ewes lambing that were bred to Cotswold and Lincoln rams were 64.8 and 68.7, respectively. Table 2 shows that these two breeding groups were significantly lower than the Romney or Columbia breeding groups in this respect. The crossbred ewes in this study originated from Romney x Navajo and Corriedale x Navajo crosses. Grandstaff (1949) found that the percentages of ewes lambing of ewes bred were 84.1 and 88.8, for the Corriedale x Navajo and Romney x Navajo crosses, respectively. However, only very limited conclusions can be made regarding the low percentages of ewes lambing when mated to the Cotswold and Lincoln rams used in this study, for the following reasons: (1) Rams of the four breeds used were not all mated to the same ewes, (2) the number of rams of each breed used was small, and (3) semen tests indicated that the semen quality of three of the Cotswold and Lincoln rams was too poor to warrant their use and in others the quality was not high. However, because of the limited number of rams available, some of the undesirable ones were used. During the 3-year period, no Cotswold or Lincoln ram settled more than 84.2 percent of the ewes allotted him. The lowest percentage of ewes settled was 11.1. These breeding groups have been consistently lower than the average of the flock each year, yet a majority of the ewes going out of these pens and into the flock after the regular breeding season were range bred and produced lambs. The Cotswold rams were obtained from three and the Lincoln rams from two different breeders. Rams of the four breeds were maintained under the same management and environment during the experiment. The low fertility of the Cotswold and Lincoln rams in this study, as was shown by the semen test, may indicate that they were adversely affected by the environmental conditions of the arid Southwest.

In fecundity no significant differences were found between the various breeding groups as shown in table 2. The average for all ewes, 141.4 percent, compares favorably with 123.4 percent in Rambouillet sheep (Terrill, 1949). On the southern ranges of Utah, mature ewes bred to Columbia rams and Rambouillet rams had 138.6 and 123.9 percent lamb crop, respectively, (Bell *et al.*, 1948).

For percentage of live lambs of lambs born, the Romney and Lincoln crosses proved superior. Table 2 shows both of these crosses were significantly better in this respect than the Columbia and Cotswold crosses. There were no significant differences between the various breeding groups for percentage of lambs weaned of live lambs born.

Mean differences between the 4 breeding groups for weaning weights of lambs and pounds of lamb produced per ewe bred are given in table 3. Size of lambs at weaning was used as a measure of growth rates between the breeding groups. Significant differences were measured by the "t" test (Fisher, 1941).

All weaning weights were adjusted for age of dam, type of birth, type of rearing and age of lambs in days (Sidwell and Grandstaff, 1949). All differences in favor of the Cotswold group, as compared with the remaining groups, for average weaning weights of lambs were

TABLE 3. MEAN DIFFERENCES BETWEEN CROSSES FOR WEANING WEIGHT OF LAMBS AND POUNDS OF LAMB WEANED PER EWE BRED FOR 1947, 1948 AND 1949

Characteristic	Columbia cross minus Romney cross	Columbia cross minus Lincoln cross	Columbia cross minus Cotswold cross	Romney cross minus Lincoln cross	Romney cross minus Cotswold cross	Lincoln cross minus Cotswold cross
Weaning weight, pounds	+2.62**	−0.71	−1.11*	−1.91**	−3.73**	−1.82**
Pounds lamb weaned per ewe bred	−0.89	+12.45**	+17.24**	+13.34**	+18.13**	+4.79**

* denotes significance at the .05 level.
** denotes significance at the .01 level.

significant. The average weaning weights of the Columbia and Lincoln groups were significantly greater than the average weaning weight of the Romney group. The mean difference between the Columbia and Romney groups for pounds of lamb weaned per ewe bred was small. The Lincoln group was significantly better than the Cotswold group for this measurement. The Lincoln and Cotswold groups were considerably poorer than the Columbia and Romney groups.

Summary

This was a study to observe differences in lamb production between some of the crossbred breeding groups maintained at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico.

Breeding and lambing records were analyzed for 721 matings of Navajo ewes with Romney and Columbia rams, and 974 matings of Navajo crossbred ewes with Cotswold and Lincoln rams.

In crosses involving Columbia and Romney rams with Navajo ewes, the percentages of ewes lambing were 88.3 and 90.2, respectively. The percentage of Navajo crossbred ewes lambing that were bred to

Cotswold and Lincoln rams were 64.8 and 68.7, respectively. However, only very limited conclusions can be made regarding the low percentages of ewes lambing when mated to the Cotswold and Lincoln rams used in this study, for the following reasons: (1) Rams of the four breeds used were not all mated to the same ewes, (2) the number of rams of each breed used was small, and (3) semen tests indicated that the semen quality of three of the Cotswold and Lincoln rams was too poor to warrant their use and in others the quality was not high. However, because of the limited number of rams available, some of the undesirable ones were used.

In fecundity no significant differences were found between the various breeding groups. The average for all ewes was 141.4 percent. For percentage of live lambs of lambs born, the Romney and Lincoln crosses proved superior. There was no significant difference among the various breeding groups for percentage of lambs weaned of live lambs born.

The Cotswold cross lambs were significantly heavier at weaning age than lambs of the remaining breeding groups. The average weaning weights of the Columbia and Lincoln groups were significantly greater than the average weaning weight of the Romney group. The mean difference between the Columbia and Romney groups for pounds of lamb weaned per ewe bred was small. The Lincoln group was significantly better than the Cotswold group for this measurement while both the Lincoln and Cotswold groups were considerably poorer than the Columbia and Romney groups.

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GENETIC AND ENVIRONMENTAL FACTORS AFFECTING STAPLE LENGTH IN NAVAJO AND NAVAJO CROSSBRED WEANLING LAMBS[1]

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STAPLE length is one of the important characteristics in determining the value of wool since it influences the price per pound as well as the amount of clean wool in a fleece. Pohle and Keller (1943) found that with each centimeter increase in staple length there was an average increase of ½ to ¾ pound of unscoured wool, ¼ to ½ pound of bone dry scoured wool per fleece, and .85 to 2.52 percent in clean yield.

Results reported by Pohle (1942) show that staple length at weanling age has a high relationship with that at yearling age. This work indicates that lambs at weanling age may be selected or culled on fleece length with a reasonable degree of accuracy. However, most characteristics are influenced by environmental factors that tend to conceal genetic merit, and corrections or adjustments for these environmental effects increase the accuracy of selection.

Hazel and Terrill (1945 and 1946) found that selection for staple length in range Rambouillet, Columbia, Corriedale, and Targhee lambs was affected by sex, age of dam, year of birth, type of birth and rearing, and age at weaning.

The purpose of this study was to determine the importance of several environmental sources of variation as they affect staple length in weanling lambs, and to obtain estimates of differences in staple length between lambs produced in the several breeding groups maintained in the breeding program at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico. The environmental factors studied were year of birth, age of dam, type of birth and rearing, and sex. The breeding groups studied were:

1. Navajo rams x Navajo ewes.
2. Romney rams x Navajo ewes.
3. Columbia rams x Navajo ewes.

[1] The data for this study were collected at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, under authority of the Bankhead-Jones Act, by the Bureau of Animal Industry, U. S. Department of Agriculture, in cooperation with the Bureau of Indian Affairs, U. S. Department of the Interior and the New Mexico Agricultural Experiment Station.

[2] U. S. Department of the Interior and collaborating with the U. S. Department of Agriculture.

4. Lincoln rams x ewes ½ Navajo, ¼ Corriedale, ¼ Romney.
5. Cotswold rams x ewes ½ Navajo, ¼ Corriedale, ¼ Romney.
6. Rams ½ Navajo, ¼ Corriedale, ¼ Romney x ewes ½ Navajo, ¼ Corriedale, ¼ Romney.
7. Rams ½ Cotswold, ¼ Navajo, ⅛ Corriedale, ⅛ Romney x ewes ½ Columbia, ½ Navajo.
8. Rams ½ Columbia, ½ Navajo x ewes ½ Cotswold, ¼ Navajo, ⅛ Corriedale, ⅛ Romney.

These breeding groups are also referred to by their respective numbers, 1 to 8, in tables 1 and 2.

Description of Data

The data used in this study were on a total of 1618 lambs, representing all lambs weaned for 2 years in the 8 groups outlined above. In 1947 the lambs were born in April and in 1948 they were born in May. To eliminate differences in staple length due to differences in ages of the lambs within a year, a small area on the left side of each lamb was clipped close to the skin when the average age of lambs was one month. At weaning time a small sample of wool was then taken from this area, and from this sample the staple length was determined. In 1947 this sample represented a total growth of 111 days and in 1948 a total of 84 days on all lambs. Only four ages of dam groups were considered in this study namely, 2 years, 3 years, 4 to 7 years, and 8 years or more.

Method of Analysis

To test the accuracy of measuring staple length using the procedure described above, correlation coefficients were computed between actual age of the lambs in days and staple length at a constant age of 84 days for all lambs weaned in 1948. The ram and ewe lambs were handled separately, and the value of r was .14 in both cases. A correlation value of .14 is sufficiently large to be considered significant at the .01 level, and indicates there is a more rapid increase in staple length among the older lambs. This situation cannot be clarified from the data at hand. Even though a significant correlation coefficient was obtained, the procedure of clipping the lambs seems justified inasmuch as it eliminates the errors involved in the task of mathematically adjusting for days of age, and also eliminates all differences in the staple length in the birth coats of the lambs.

Hazel and Terrill (1945) found that in range Rambouillet lambs staple length must increase at an average rate of about 0.02 centimeter

per day from birth to weaning, in order to attain the observed average length of 2.66 centimeters at 124 days of age. These workers concluded that length of staple as measured in these lambs did not increase linearly with age. Thus it is these authors' belief that a single regression figure with which to make adjustments for age on all lambs could not be used accurately, and clipping the lambs at a very early age encounters no greater error than would be found in measuring the total staple length from birth to weaning.

TABLE 1. NUMBERS OF LAMBS WEANED, ASSEMBLED ACCORDING TO BREEDING GROUPS AND ENVIRONMENTAL FACTORS STUDIED

	Age of dam				Sex		Type of birth and rearing			Years	
Breeding Group	2 years	3 years	4–7 years	8 years and older	Rams	Ewes	Singles	Twins	Twins raised as singles	1947	1948
1	27	2	142	94	125	140	74	170	21	135	130
2	53	85	88	10	110	126	104	114	18	109	127
3	39	47	157	3	110	136	113	118	15	131	115
4	40	54	258	34	175	211	138	212	36	135	251
5	17	34	125	7	89	94	73	90	20	87	96
6	35	29	163	11	108	130	83	139	16	238	0
7	34	0	0	0	14	20	25	9	0	0	34
8	30	0	0	0	12	18	27	2	1	0	30

The number of lambs weaned are listed in table 1 according to their various classifications. Numbers 1 to 8 in the first column of table 1 indicate the various breeding groups as outlined previously in this report. The method of analysis used in this study is the least squares method suggested by Yates (1934) and extended by Hazel (1946) and Henderson (1948). This method consists in fitting constants for each of the independent variables, which in this case are the measurable environmental factors and different breeding groups. This method was designed for the application of analysis of variance to data where multiple classification with unequal numbers in different classes is encountered.

Table 2 shows the estimates of the deviations for each subclass of the major classification. Table 3 shows that variations due to breeding groups, to years, and to sex are highly significant while differences due to type of birth and rearing and to age of dam are not statistically significant. Since type of birth and rearing and age of dam were not significant, new least squares equations were solved leaving out these two

sources of variations. The new estimates of the deviations with the non-significant constants eliminated are also found in table 2.

The total sum of squares associated with the 1617 degrees of freedom was 8566, while the total reduction associated with the 14 degrees of freedom was 3068. Thus about 36 percent of the variance in staple length in these lambs is due to the measurable environmental factors

TABLE 2. SUMMARY OF ESTIMATE OF DEVIATION FROM MEAN SHOWING THE VALUE FOR EACH SUBCLASS OF THE MAJOR CLASSIFICATION

Major classification	Subclass	Estimate of deviation	Estimate of deviation with non-significant constants eliminated
Breeding group	1	—2.13	—2.08
	2	—0.78	—0.81
	3	—0.32	—0.36
	4	0.89	0.88
	5	1.61	1.59
	6	0.64	0.62
	7	0.04	0.07
	8	0.05	0.09
Age group of ewes	2 years	.03	
	3 years	—.10	
	4–7 years	—.12	
	8 years older	.08	
Sex	Rams	—.29	—.29
	Ewes	.29	.29
Type of Birth and rearing	Singles	.00	
	Twins	.01	
	Twins raised as singles	—.02	
Years	1947	.63	.64
	1948	—.63	—.64

and group differences. Hazel and Terrill (1945 and 1946) reported about 18 percent of the variance accounted for in range Rambouillet lambs, and about 12 percent in Columbia, Corriedale, and Targhee lambs.

The mean square for error in table 3 is 3.4 indicating that the standard deviation for staple length is about 1.85 centimeters in the data adjusted for the environmental factors and group differences, as compared with 2.32 centimeters in the unadjusted data.

Results and Discussion

Fleece improvement through selection is limited because only part of the observed variation between animals is due to genetic differences. Other differences are caused by environmental factors which may be corrected or adjusted to the extent that their effect can be accurately measured. Thus progress in staple length improvement through selection can be increased in so far as the staple length differences can be accurately corrected for inequalities due to environmental factors.

Of the sources of variation studied only differences due to breeding groups, sex, and years proved to be sufficiently large to warrant adjust-

TABLE 3. ANALYSIS OF VARIANCE FOR ENVIRONMENTAL FACTORS AND BREEDING GROUPS

Source of variance	Degrees of freedom	Sum of squares	Mean squares
Total	1617	8566	
Total reduction due to fitting all constants	14	3068	
Between breeding groups	7	2116	302.4**
Between ages of ewes	3	3	1.0
Between sexes	1	141	141.8**
Between types of birth and rearing	2	2	1.1
Between years	1	494	494.4**
Remainder	1603	5497	3.4

** Indicates highly significant mean squares.

ments. Differences due to age of dam and type of birth and rearing were not statistically significant. Yearly differences were large because the staple length of lambs born in 1947 represented a 4-weeks' longer growth period than in 1948. However, Hazel and Terrill (1945) found a significant difference in staple length between years for lambs born during the same month in two different years.

Ewe lambs averaged 0.59 centimeter longer staple than ram lambs. Studies with other data at this station show a similar sex difference in other years, indicating that this difference is not peculiar to the two years involved in this study. The reverse of this difference is observed with the yearling ewes and rams at this station. However, the rams are a more highly selected group than the ewes, and such a comparison in staple length is not wholly justified. Hazel and Terrill (1945 and 1946) found similar sex differences of 0.48 centimeter in range Rambouillet

lambs and 0.54 centimeter in Columbia, Corriedale, and Targhee lambs.

Breeding group differences, although not considered as environmental variations, show a range in staple length from 7.77 centimeters in the Cotswold sired crossbred lambs to 4.11 centimeters in the Navajo lambs.

Summary

The effects of breeding groups, sex, age of dam, year of birth, and type of birth and rearing on staple length were studied on 1618 lambs, representing all lambs weaned in 1947 and 1948 at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico.

The effect of age at weaning on staple length was held constant by clipping an area close to the skin when the average age of the lambs was one month. In 1947 this growth period represented 111 days on all lambs, and in 1948 a period of 84 days' growth on all lambs.

Variations in staple length due to age of dam and type of birth and rearing were not significantly different, indicating adjustment need not be made for these factors. Ewe lambs averaged 0.59 centimeter longer staple than did the ram lambs. Breeding groups varied from an average of 7.77 centimeters in Cotswold sired crossbred lambs to 4.11 centimeters in the Navajo lambs.

The factors studied accounted for approximately 36 percent of the total variation in staple length. If the more important differences due to environmental factors are considered in culling lambs, more improvement from selection can be expected.

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RECORD OF THE PROCEEDINGS OF THE FORTY-SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

THE forty-second annual meeting of the American Society of Animal Production was held in the Hotel Sherman in Chicago on November 24 and 25, 1950, and a dinner for the honor guest was held at the Stockyard Inn on November 26. Abstracts of most of the papers presented were published in the November 1950 issue of the JOURNAL OF ANIMAL SCIENCE, volume 9, pages 634–684.

PRESIDENT'S ADDRESS

P. S. SHEARER

President, American Society of Animal Production

IT is my privilege and pleasure to welcome you this morning to the opening session of the forty-second meeting of the American Society of Animal Production. Since the general theme of the morning program is in part historical it seemed appropriate to review a little of the development and the present status of the Society. This review is all the more timely because one of the major problems facing us today as an organization stems from the extremely rapid growth which we have experienced in the recent post-war years.

According to the records, the Society was conceived at an informal meeting of Nutrition workers gathered for a graduate school at Cornell University in 1908. At that meeting a committee was appointed to survey the field and prepare recommendations that would lead to improved quality of investigations in animal nutrition and better cooperation among the various experiment stations. Arrangements were made for these recommendations to be presented at a called meeting of Experiment Station workers in Animal Husbandry in Chicago in November, 1908. It is recorded that eighteen states and the United States Department of Agriculture were represented at that meeting by a group of thirty-three men and that a formal organization to be known as the American Society of Animal Nutrition resulted from their deliberations.

It soon became clear to the members that the scope of the Society should not be confined to the field of Animal Nutrition and in 1912 the

name was officially changed by substituting the word "Production" for "Nutrition." At the same time the guiding objective was revised to read "improve the quality of investigations in animal production and promote better correlated studies in breeding, feeding and management of the different classes of livestock."

Up to this time emphasis was on research and the base had been broadened to include all phases of Animal Husbandry investigations. Another important milestone came in 1915 when the objectives were again revised to read as follows "to bring about improvement and unification of methods of investigation and ***instruction*** in animal production." The word "instruction" was immediately interpreted to include extension and later the word "extension" was written into the objective.

Membership numbers increased from the original thirty-three charter members to reach a rather stable figure of around 400 during the late twenties and all through the thirties. By 1946 the number had increased to 694 and in 1949 to 1044. I am advised by the secretary that membership for 1950 is substantially above that reported last year. Attendance at the annual meeting has shown a pattern of growth similar to that of membership numbers with a striking increase in recent years as indicated by these figures.

Year	Attendance
1931	135
1936	180
1946	452
1949	633

With this increase in scope of activity, membership numbers and attendance has come a marked increase in the number of papers submitted for presentation at the annual meetings. Since the first objective of the organization as stated in our present constitution is to afford opportunity for the exchange of ideas in the field of interest of the Society it seems obvious that a major responsibility of the Society is to provide the very best possible forum to accomplish this worthy objective.

At the early meetings this was not a problem. Only eleven papers were presented for the first five sessions. All members met together and had the opportunity to hear all of the papers and there was ample time for discussion. In 1925 there were fifty papers, in 1940 there were seventy-four and in 1949 a total of 151 papers (including 22 to be read by title only) were listed on the official program. As the number of papers submitted increased a problem developed which was met by

sectioning the group, with various section programs running concurrently, which is our present practice.

With respect to programs for the annual meeting your Executive Committee is currently working under a directive recommended by a Program Committee and accepted by the Society at the 1946 meeting. It reads as follows:

"That the sectional programs be based principally upon the general classification: Animal Nutrition, Animal Breeding and Genetics, Animal Physiology, Extension, and Teaching.

That sectional programs be scheduled probably as follows: Animal Nutrition on Friday afternoon, Friday evening, and Saturday morning; Animal Breeding and Genetics and also Animal Physiology on Friday afternoon and Saturday morning; Extension and also Teaching on Friday evening.

It is recognized that some flexibility in programs and in schedules may be necessary. Therefore, it is recommended further, that the Executive Committee of the Society be empowered to make modifications, and to make provisions for a sectional program on Livestock Management and Economics and/or for symposiums upon special subjects as considered advisable.

A meats section was authorized by special action at the same meeting.

Criticisms of the meetings have been frequent in recent years and the problem of improving them is not a simple one. Critics of the present plan for conducting the meetings should study the alternatives. Some of the alternatives that have been frequently suggested are (1) a three-day session, (2) more sections, and (3) fewer papers. This is not the place to evaluate these alternatives except to say that there are valid objections to each of them. Every member should be aware of the problem and be assured that the Executive Committee welcomes constructive suggestions.

There has also been a large increase in recent years in the number of papers submitted for publication in the Journal of Animal Science. This has placed a heavy and increasing burden on the Editor. It is the judgment of the present Executive Committee that a way must be found to relieve him of some of his present responsibilities.

Our surplus fund has been growing in recent years and amounted to $16,787 in 1949. In the early years the policy was to have sufficient income to cover the actual expenditures from year to year. That this job was well done is indicated by an accumulated surplus of $7.28 in 1926 after operating for eighteen years. Since then, with an increased membership, the surplus has grown to the figure given above. A surplus fund can be accumulated for emergency use or it can be built up for some designated purpose other than to safeguard regular operating expenses. What is ours for? If for emergency use only, is it large

enough now? There is no good reason for accumulating a surplus beyond what is needed. This is obviously not a pressing problem but all members should be aware of the situation.

I want at this time to thank all of the officers, committees and members who have contributed during the year to the conduct of the affairs of the Society. Probably the two members who give most in time and effort are our secretary and our editor. To them should go the special commendations of this group for work well done.

The program for this morning session has been arranged with the thought that this mid-century year of 1950 would be an appropriate time to take a few moments from our present activities and look back over the road we have travelled. The art and science of Animal Husbandry has made much progess since 1900. Members of this Society and the influence of the Society itself have played an important role in that progress. An appraisal of what has been done should be of interest, particularly to the younger members, and helpful to all of us in charting our course in the years that lie ahead.

A SUMMARY OF THE MINUTES OF THE FORTY-SECOND BUSINESS MEETING OF THE AMERICAN SOCIETY OF ANIMAL PRODUCTION

H. M. Briggs, Secretary

University of Wyoming

THE meeting was called to order in the Louis XVI Room of Hotel Sherman, Chicago at 2:00 p.m. by President Shearer. The minutes for the 1949 meeting were not read as they had been published in the Journal of Animal Science, Vol. 9(1):119–124. It was moved that they be approved as published and the motion carried.

The secretary read a report which showed the domestic membership to be 1,077 and foreign membership 48. This is an increase of 92 domestic members and a loss of 11 foreign members. Three circular letters were sent to the membership during 1950. During the year a new contract was made with the Boyd Printing Company, Albany, New York, to publish the Journal after January 1, 1951. There will be a very slight reduction in printing costs for the Journal, but a slight increase in printing costs for reprints.

The treasurer's report showed that during the year $1100.00 had been spent from the Morrison Award Fund, leaving a balance of $1291.10. Interest accrued on the Morrison Award Trust Fund as of December 31, 1949, was $1416.67. There were receipts totaling $11,121.83 received by the Society against an expenditure of $10,016.13 or there was an increase of $1105.70 above operating expenses. The General Fund shows a tangible cash balance of $4528.81. In addition, the Society has assets of $4550.70 in U. S. Bonds, Series F, and $7000.00 in U. S. Bonds, Series G.

The Auditing Committee, consisting of J. L. Lush, A. D. Weber and Paul Gerlaugh, found the treasurer's books in satisfactory order.

The editor of the Journal of Animal Science, L. E. Casida, presented the following statements to the membership:

"A total of 590 papers have been submitted for consideration for publication in the Journal of Animal Science from its beginning up through November 15, 1950. Of these, 473 have been published in volumes one to nine inclusive.

"Publication during 1950 included 85 papers, the president's address and other Society proceedings of the 1949 meetings, 144 abstracts of papers to be presented at the 1950 annual meeting of the Society, subject and author indexes of Volume 9

and the usual News and Notes section in each of the four issues of the JOURNAL.

"Manuscripts received during the period November 16, 1949 to November 15, 1950, totaled 129. Nine of these were combined into two papers of four and five parts, respectively, covering two symposia given at the Society meetings in 1949. This gives for consideration 122 papers. Of these 3 were withdrawn or rejected, 55 have been published, 32 are in press to appear in the February 1951 issue, 9 others are edited and ready for the printer, 7 are now being reviewed by the Editorial Board, and 16 others are in the process of being revised by the authors. (It might be mentioned here that of the nine papers reported under revision by the authors a year ago, six have not yet been accepted for publication.) A maximum of 96 acceptable papers became available for publication during the past year and at the same time 85 papers have been published in Volume 9 of the JOURNAL. This discrepancy reflects the increasing rate at which manuscripts were being received during the course of the year. All papers on which editorial work had been completed at the time, were sent to press for each of the four issues. Publication is, therefore, as nearly current as possible.

"The number of papers published in each of volumes 1 to 7 of the JOURNAL ranged from 39 to 55. In Volume 8 there were 63 and in the current volume there are 85—an increase of more than 1/3 in one year. The total number of manuscripts processed during the past year, 129, can be compared with 72 a year ago and is an increase of more than ¾. The Society should be cognizant of the marked rate at which the publication load of the JOURNAL, and its attendant costs, are increasing and of the enlarging demands which are being made of the Editorial Board in processing the growing volume of manuscripts submitted for publication. Appropriate action to relieve excessive demands on individuals of the Board and to appraise our position relative to the cost of the increased load of publication needs to be given by the Society at an early date."

The report of the business manager of the JOURNAL OF ANIMAL SCIENCE showed the following numbers of the JOURNAL available:

Volume	Year	Complete volumes	Copies of numbers in addition to complete volumes			
			No. 1	No. 2	No. 3	No. 4
1	1942	0	0	31	38	57
2	1943	34	39	24	0	9
3	1944	0	3	0	0	0
4	1945	0	0	0	0	0
5	1946	0	0	0	0	0
6	1947	0	0	0	0	0
7	1948	33	3	0	13	0
8	1949	0	0	0	46	40
9	1950	0	0	0	125	125

These back numbers are for sale by the Business Manager of the JOURNAL. Single copies are $1.00 in the United States. A complete volume is $3.50. Prices elsewhere quoted on request. In addition, the following copies of the Proceedings of the American Society of Animal Production are available.

Year	No.	Year	No.
1915–16	60	1930	32
1920	10	1931	76
1922	370	1932	30
1923	245	1934	50
1924	0	1935	51
1925–26	64	1937	0
1927	62	1938	0
1928	0	1939	21
1929	44	1940	76

The report of the Nomination Committee was published in the November, 1950 issue of the JOURNAL OF ANIMAL SCIENCE. In the election that followed, E. W. Crampton was elected President, H. M. Briggs, Vice-President, and J. I. Miller, Secretary-Treasurer.

Clair E. Terrill, Chairman of the Investigations Committee gave the following report:

"Former committees on investigation and the Society have repeatedly favored the publication of a monograph. This year your committee has attempted to prepare tentative plans for the publication of a monograph for the Society to act on.

"The topic proposed is 'Techniques and Procedures in Animal Production Research'. This would be a sort of handbook on animal husbandry research. The objective is to assemble in one volume the information on research techniques which are important and generally unique to farm animals. The monograph would be divided into 5 sections as follows: (1) general research methods (including some statistical procedures), (2) animal nutrition, (3) animal breeding, (4) physiology, and (5) meats. Authors have been chosen and contacted but with the provision that manuscripts would not be prepared unless and until the Society approves the plan.

"Publication of about 2500 copies similar in size to one issue of the JOURNAL OF ANIMAL SCIENCE is planned. An estimate of cost is about $1.32 per copy. It is recommended that such a monograph be published by the Society and that an assessment of the cost per copy plus 25 per cent be added to the dues of each member for 1952. The remainder of the cost would be borne by the Society and the remaining copies would be sold at approximately cost plus 50 per cent."

F. B. Morrison raised the question of an extra number of monographs being published so that the "parentage" of the monograph would not be lost. W. G. Kammlade made a motion that the secretary take a ballot of the Society to see how many would be interested in buying a copy or having their dues raised, in order to determine the advisability of undertaking the publication. E. H. Hughes seconded the motion. Comments followed by F. B. Morrison, R. W. Phillips, and L. E. Casida, after which the motion carried.

E. H. Hughes, chairman of the Instruction Committee, reported as follows for his committee:

"The Committee on Teaching, at your request, presented a discussion of animal husbandry curricula at this year's meeting. We believe that graduate study in animal husbandry is becoming increasingly important in our universities and colleges and that a discussion of the Master's degree in animal husbandry would be an appropriate project for next year's session of the Society. Your committee recommends that this be done. The Extension Committee gave no formal report."

D. W. Colvard read the following report for the Pasture and Range Investigations Committee:

"Your committee on pasture and range investigations in cooperation with the pasture committees of the American Society of Range Management, American Society of Agronomy and the American Dairy Science Association has nearly completed the revision of the report on pasture and range investigations. The completed report will be ready for publication during 1951. Steps are being taken to publish the report in a journal of one of the four societies.

"Your committee on pasture and range investigations was carried over into 1950 to complete the above report. It is understood that this committee will be discharged upon the completion of the report.

"It is recommended that a new pasture and range investigations committee be appointed for 1951 to consider other problems in this field. We wish to recommend that the new committee join with pasture committees of other societies in making the necessary recommendations, and taking the necessary steps to bring about improvement in methods of enumeration of census data pertaining to pastures."

It was mentioned that the existing committee would complete the supervision of the above mentioned report through publication.

C. W. Gay, Chairman of the History of Livestock Committee gave the following report:

"Copy of the first draft of the material on the history that has been prepared to date, together with a synopsis of the complete coverage in subject matter contemplated in the finished work has been circulated among the members of the committee for their consideration and criticism.

"The program for the one year remaining of the four-year assignment includes completion of subject matter as well as a full bibliography. The finished manuscript revised as suggested by the committee will be presented at the annual meeting of the Society in 1951.

"Considerable reference material is being accumulated for the use of the Society."

The following report by the Committee on Information was read by the secretary:

"The work of the committee covering this current meeting is just beginning. News stories covering a number of the papers presented are now being released to the livestock and meat industry press and other news services. For this reason, this committee's report as usual, will cover the results of the committee's activities in connection with the 1949 Society meeting.

"News stories emanating from the 1949 meeting have come to our attention in 54 different publications of 22 different states, ranging from New York state

to California. These stories appeared in 31 papers of the industry—including market, breed, general livestock, farm and meat trade publications. Stories also appeared in 23 daily papers. The space represented by these stories is the equivalent of 36 newspaper columns.

"The experience of the committee shows that news stories must cover practical facts with a minimum of scientific data, valuable though the scientific data may be —to win the approval of editors.

"It is of interest to note that some of the news stories concerning last year's meeting were used even as late as June and July of this year. This fact showed the desire of the editors to use such material even though at a late date."

It was moved that the committee be especially commended for its services. The motion was seconded and carried.

E. F. Ferrin, chairman of the Necrology Committee, presented the names of the members who passed away during the year. Eulogies are published in the current issue of the JOURNAL. Members stood in silent respect.

J. C. Miller, chairman of the Beef Cattle Type Specifications Committee read the prepared statement:

"The purpose of this committee was to consider the adoption of suitable definitions of types or sub-types to guide research workers making within-breed comparisons and to make recommendations accordingly.

"Your committee is agreed that there is a need for type standards or specifications for use by research workers in order to promote greater uniformity in the reporting and interpretation of results of type studies.

"This committee feels there is insufficient data available at present on which to base reliable type specifications. Research projects are now underway in several states, particularly in Colorado, Oklahoma and Texas which will shed light on this question in the near future.

"Your committee recommends to the Society than an invitation paper summarizing existing data on beef cattle type studies be included on our 1951 program."

The following resolutions were presented by the committee and were passed by the Society:

1. To the management of the Hotel Sherman, we express our thanks and appreciation for rooms and facilities provided for our meetings without cost to the Society.

2. To John Morrell and Company, we are indebted for the splendid service rendered in making available at our meetings reprints of the abstracts of the papers presented. We are grateful for this interest in the work of the Society.

3. To the officers and members of program committees we extend commendation for the excellent programs presented to those attending the 1950 meetings.

4. To Frank B. and Elsie Morrison and to the American Feed Manufacturers' Association, we express our sincere thanks for the meritorious awards they have made available to the Society. These awards have and will in the future greatly stimulate research work.

5. Due to requests from members of the Society, we recommend that the Executive Committee of the Society consider canvassing members again as to whether the Society should not hold their annual meetings at different locations from year to year and that the time of the meetings should be during the latter half of June.

E. A. Livesay, Society representative to the Inter-Association Council, had nothing to report, since no meetings had been held.

The secretary read the following report by H. C. McPhee, Society representative to the Inter-Society Committee on Science Foundation Legislation:

"Since the last meeting of our group, the Science Foundation Legislation has become law and the Inter-Society Committee has been disbanded. There is no longer any need of the special representation for this activity."

F. F. McKenzie, Society representative to the American Association for the Advancement of Science Council, gave the following report:

"Representatives from affiliated societies of the A.A.A.S. make up the Council. These elect the Executive Committee of the A.A.A.S.

"Few matters came up during 1950. The Council invites material such as articles and news items from A.S.A.P. members, such to be published in one of the two journals, Science or Scientific Monthly.

"Your representative deems it worthwhile to retain our connection with A.A.A.S., especially in times of national emergency when we might be helpful on such problems as food, manpower and techniques."

H. W. Marston, Society representative to the Division of Biology and Agriculture, National Research Council, gave the following report of the Division meeting held last May:

"The Executive Committee of the Division agreed that member societies should be solicited for advice and suggestions regarding specific problems of interest to the societies in which the Division could be of assistance.

"The Committee on Animal Nutrition reported the following progress on reports.

1. New—Recommended Nutrient Allowances for Horses.
2. Revised—Recommended Nutrient Allowances for Sheep.
3. In Press (Revised)—Recommended Nutrient Allowances for Poultry, Dairy Cattle and Swine.
4. In Progress—Recommended Nutrient Allowances for Beef Cattle.

"Three new committees have been set up on Recommended Nutrient Allowances for dogs, rabbits and fur-bearing animals.

"The published reports have been extensively used in improving the feeding of animals and by the feed manufacturing industry.

"The Feed Composition Committee reported progress in the development of feed composition tables, but lack of funds had stymied further progress. An attempt is being made to work out cooperation with the U.S.D.A. for completing the work.

"The American Institute of Biological Sciences reported that considerable prog-

ress had been made on the preparation of the 'Handbook of Biological Data' and it was anticipated that the manuscript would be in the hands of the printer by September, 1951.

"The Committees on Fulbright Fellowships screened 130 applications during the year. Of this number the Conference Board Committee recommended that 32 fellowships be granted. Of interest to this Society was the awarding of the following: Biochemistry and Nutrition, 2; Genetics, 2; and Physiology and Pharmacology, 10. Eleven countries participated in the program: Belgium and Luxembourg, Burma, France, Greece, Italy, The Netherlands, New Zealand, Norway, Philippine Islands, United Kingdom, and the United Kingdom Colonial.

R. W. Phillips, Society representative to the European Animal Husbandry Association reported as follows:

"The European Association for Animal Production was founded formally in October 1949 when the agreement setting up the Association was signed in Paris by representatives of animal production organizations in Austria, Belgium, Denmark, Franch, Germany, Iran, Italy, The Netherlands, Spain and Switzerland. The Italian Breeders Association put at the disposal of the Association an office situated at Via Quintino Sella 45, in Rome, and agreed to make available the necessary furniture and office equipment.

"The Council of the Association held its first meeting in Zug, Switzerland, on December 11, 1949, and appointed H. Aerse as Secretary-General. Mr. Aerse took a year's leave from his assignment in Denmark to carry out this work for the Association. The Council also set up six committees to deal with: (1) Coordination of research (Mr. Braconnier of France, Chairman); (2) Animal nutrition (Prof. E. Crasemann of Switzerland, Chairman); (3) Cattle breeding and production (Mr. Th. C. J. M. Rijssenbeek of The Netherlands, Chairman); (4) Horse breeding (Mr. L. Rulot of Belgium, Chairman); (5) Sheep and goat breeding (chairman being selected); and (6) Pig breeding (chairman being selected).

"The Council held another session at Ghent, Belgium, on October 10–14, 1950, and during this period convened a Study Meeting to discuss the incidence and use of hormones in animal production. Papers were submitted by various specialists engaged in research on this subject, including Prof. John Hammond of Cambridge University, England, Dr. R. M. Fraps, of Beltsville, Maryland, Dr. R. Moustgaard of Denmark, and Prof. Simmonet and M. le Bars of France. In addition a paper by Prof. J. W. Amschler of Austria was circulated but not read. Approximately 150 workers from 12 European countries participated in this Study Meeting. In view of the success of the Study Meeting, the Council decided to organize a second such meeting in 1951 and accepted an invitation from the Dutch member of the Association to hold it in the Netherlands, the tentative date being July 15–21. The topic will be 'Progeny testing.'

"Much attention was necessarily given by the Association, during its first year, to organizational and financial matters, and the Council is still faced with the problem of finding a permanent Secretary-General. However, it appears that a good foundation has been laid and that in the future the Association should provide an effective forum for discussion of animal production problems common to many countries in Europe."

H. R. Guilbert moved that "The Executive Committee consider the problem of appointing a special committee to consider the importation of livestock to the United States and recommend a stand on the question to the Society." F. F. McKenzie seconded the motion. B. T. Simms, U.S.D.A. made a statement relative to the importation of livestock and carcasses and commented that Congress is the only body that can change existing laws. The motion carried.

The meeting adjourned at 4:00 p.m.

MEMORIALS

CHARLES I. BRAY

Charles I. Bray was born at Huntsville, Ontario, Canada, July 29, 1882, and died March 23, 1950, at Baton Rouge, Louisiana. He graduated from the Ontario Agricultural College in 1904 and received a Master of Science degree from Mississippi Agricultural and Mechanical College three years later. Pursuing further graduate work at both the University of Missouri and the University of Illinois he earned his Doctorate at the latter institution in 1926. Dr. Bray began his professional career at Mississippi A. and M. where he was employed in dairy husbandry work from 1904–10. From 1910 to 1914, he was assistant professor of animal husbandry at the Oklahoma Agricultural and Mechanical College. He served first as assistant professor and later as associate professor of animal husbandry at the Colorado Agricultural College from 1915 to 1927. From 1927 to 1931, he held the position of professor at Louisiana State University and was also station animal husbandman. From the latter date to the time of his death, Dr. Bray was research professor and head of animal husbandry research at the Louisiana Experiment Station.

In addition to the authorship of many bulletins, Dr. Bray served as livestock editor of the "Oklahoma Farmer Stockman" and livestock advisor of "Western Farm Life." He was also a co-author of the text book "Practical Activities in Animal Husbandry." He was a past president of Lambda Gamma Delta, and a vice-president of the Southern Livestock Association. Dr. Bray was a fellow of the American Association for the Advancemnt of Science; a member of the American Association of University Professors and the Louisiana State Teachers Association. He was also a member of the American Genetics Association, Alpha Zeta, Alpha Gamma Rho, Pi Gamma Mu, Block and Bridle, and Kiwanis Clubs. He was a Mason and an active member of the Methodist church, which he served as a member of the Board of Stewards and as Superintendent of the Sunday school for 12 years prior to his death.

Dr. Bray is survived by a daughter, Mrs. C. H. Rounds of Baton Rouge with whom he lived.

ALFRED B. CAINE

A. B. Caine, Professor of Animal Husbandry at Iowa State College passed away on August 18, 1950, at Mesa, Arizona. "A. B." as he was known and greeted by students, faculty and friends alike was an outstanding teacher of animal husbandry and internationally known for his knowledge and love of horses.

Professor Caine was born on a farm near Logan, Utah on September 13, 1891. He had his elementary and high school education at Logan and was graduated from Utah State Agricultural College in 1914. He entered Iowa State College as a graduate student in 1915 and was granted his Masters' Degree in 1917.

In 1916 Professor Caine was appointed to an instructorship at Iowa State College where he served long and well as a teacher and counselor to senior college students. He was advisor to the Agricultural Council for 29 years and he kept abreast of the problems of the students and always took an active personal interest in their affairs. He was deeply interested in horses and carried on a research program dealing with the management, nutrition and working ability of horses.

Professor Caine was ever and always a promoter of good horses. He was the first president of the Iowa Saddle Horse Association and was secretary-treasurer of the Iowa Horse and Mule Breeders' Association since 1941. His judging ability of horses was highly respected. He judged horses at the Sesqui-Centennial at Philadelphia, at the International Livestock Exposition at Chicago and at the State Fairs in Iowa, Minnesota, Michigan and Utah.

During his years of college study and ensuing years of interest in the field of agriculture, and especially animal husbandry, that followed, Professor Caine

gained membership in numerous scholastic, honorary and professional organizations. He held membership in Phi Delta Theta, Alpha Zeta, Gamma Sigma Delta, Phi Kappa Phi, Cardinal Key, The American Society of Animal Production and the Block and Bridle Club.

During his 33 years of teaching and 21 years as counselor of senior college students, Professor Caine had helped hundreds of students solve their college problems. He will be sorely missed and his passing is deeply mourned by his many friends.

Professor Caine is survived by his wife, Anna T. Caine, one daughter, Mrs. Jackson Bogle, and two sons, Richard T. and Alfred B. Jr.

WALLACE H. DAMERON

Wallace H. Dameron died suddenly following a heart attack on October 26, 1950. He had been superintendent of the Ranch Experiment Station near Sonora, Texas for a period of 22 years. He was born in Lynchburg, Virginia in 1896 and moved to Texas with his family at the age of two years. Mr. Dameron was in service during World War I and after his discharge attended the University of Wyoming, receiving a Bachelor's degree. He took a position at the Texas A. and M. College as a wool specialist in the year 1927, going to the Ranch Experiment Station the following year.

Mr. Dameron was one of the principal authorities on sheep and goats in the southwestern states and judged at many shows. He was in charge of an extensive program of range management and sheep improvement during his tenure at the Ranch Experiment Station. One of the principal research projects which is attracting wide attention is the progeny test for rams. This study is to determine how the desirable traits of wool and mutton sheep, such as the length of staple, quantity and quality of wool, weight gains and other characteristics can be transmitted by selective breeding.

The survivors of Mr. Dameron include his widow and a daughter, Miss Marjorie Ann of Victoria, Texas.

KENNETH MARVIN DUNN

Mr. Dunn was born July 23, 1918 near Hastings, Michigan, and died in an auto accident July 30, 1950 at Millett, Michigan. He received a Bachelor's degree at Michigan State College in 1942 and a Master's degree in 1947. At the time of his death most of the work for a degree of Doctor of Philosophy had been completed. From 1942 to 1945 he served as a combat fighter pilot in the Pacific War area, attaining the rank of Captain.

Mr. Dunn was a member of the staff in dairying at Michigan State College from January 1946 until his death. As an Assistant Professor in research, his principal contributions were studies of the nutritive values of corn silage and the role of soil fertility on the nutritive value of feeds for dairy cattle.

Mr. Dunn was a member of Sigma Xi, the American Dairy Science Association and the Farm House Fraternity. He is survived by his wife, a son and a daughter.

WALTER L. GAINES

Dr. Walter L. Gaines, 69, pioneer dairy scientist in Illinois, died November 20, 1950, in Urbana, Illinois. He retired in June 1949 after 30 years of continuous and significant service in the University of Illinois College of Agriculture and to the science of dairy production the world over.

Dr. Gaines was instrumental in organizing the first dairy herd improvement association in Illinois in October 1910. It was also one of the first in the nation.

A pioneer researcher in the physiology of lactation and milk production, Dr. Gaines received the Borden award in 1948, the highest honor accorded a scientist in

his field. The citation for this award aptly termed him "the modest leader in the United States of the scientific approach to the problem of milk secretion."

The scientist also devised and built several ingenious instruments and calculators without which these experiments could not have been performed.

Primarily self-trained in mathematics, he used this science extensively in his researches involving the physiological function of milk secretion. By use of mathematics he developed sound feeding standards and was able to differentiate between nutrients required for maintenance or milk production. His F.C.M. (Fat Corrected Milk) concept is used universally.

Among his other honors, Dr. Gaines was the official representative from Illinois to the World's Dairy Congress in Copenhagen in 1931.

He was a fellow of the American Association for the Advancement of Science and a member of the American Society of Animal Production, American Dairy Science Association, the American Genetic Association, the Society of Sigma Xi, Alpha Zeta, and Gamma Sigma Delta. During World War I, Dr. Gaines worked under federal supervision on a committee to increase food production.

A native of Crete, Illinois, Dr. Gaines received his Master's degree from the University of Illinois in 1910 and earned his Doctor's degree from the University of Chicago in 1915.

At a supper honoring Dr. Gaines just before his retirement in June 1949, Dr. G. W. Salisbury, Illinois dairy science department head, praised Dr. Gaines' work, saying: ". . . the pattern of fundamental research and devotion to human relations exemplified by Dr. Gaines will be continued by each member of the department to the best of his ability."

BANNER BILL MORGAN

Born at Bandon, Oregon, November 18, 1915, Dr. Morgan passed away at Rock Springs, Wyoming, September 8, 1950 while en route home from California. As a student at the Oregon State College, he received degrees of B.S. in 1937 and M.S. in 1938. He continued graduate work at the Iowa State College and the University of Wisconsin, being granted a Ph.D. at Wisconsin in 1941. From that year, he was a member of the staff as a parasitologist and instructor in Veterinary Science, 1941–43; assistant professor and later associate professor.

In the last ten years, Professor Morgan was the author or co-author of approximately 40 papers in the field of parasitology. He studied the parasites of both birds and mammals of North America, giving special attention recently to bovine trichomoniasis. In a short span of years he achieved wide-spread recognition as an authority on livestock diseases in his special field and made many friendships among both students and faculty of the University of Wisconsin. He was a member of Alpha Zeta, Gamma Sigma Delta, Beta Phi, Phi Sigma and Sigma Xi.

Dr. Morgan is survived by his wife of Madison, Wisconsin and his parents living in Ceres, California.

WILLIAM CHARLES SKELLEY

Death claimed Professor Skelley on September 14, 1950, following a heart attack August 23. He had been a member of the Agricultural College and Experiment Station staff of Rutgers University since 1919. Born in Cleveland, Ohio, March 18, 1896, he attended the Hopedale, Ohio schools and Ohio State University qualifying for a B.S. degree in 1918, and an M.S. in 1923. He progressed from instructor to professor of animal husbandry (1927) and animal husbandman of the New Jersey Agricultural Experiment Station. In addition to the direction of the work in animal husbandry, Professor Skelley was manager of the 1,000 acre college farm for a long period of years. He was in close touch with the livestock industry

of the state of New Jersey, serving as secretary and vice-president of the New Jersey Aberdeen Angus Association and as secretary of the New Jersey Sheep Breeders' Association.

Mr. Skelley was a director of the New Brunswick Chamber of Commerce; past president of the local Lions club; a member of the Grange, the Elks the Trenton, New Jersey Consistory and the Shrine and the Delta Theta Sigma and Lambda Gamma Delta fraternities. He was the author or co-author of numerous agricultural bulletins and journal articles. He is survived by his wife; by his mother and a brother, Lawrence, of Columbus, Ohio.

CYRIL JAMES WATSON

C. J. Watson, Head of the Animal Nutrition Unit, Division of Chemistry, Science Service, Ottawa, Canada, died on August 25, 1950 in his 49th year. Dr. Watson studied at McGill University and at Cornell, where he received his Ph.D. in 1928. He joined the staff of the Division in Ottawa in 1919 while still a student, and was associated with it until his death. Dr. Watson had a dynamic personality and was an inspiration to all who were associated with him. He had the ability to immerse himself in a problem to the exclusion of everything else. He was internationally known for his studies on digestibility of feeds for cattle, sheep and swine, which he reported in some 25 papers. These studies were characterized by the senior author's careful planning and meticulous attention to detail. Members of the Society will remember Dr. Watson's impatience at annual meetings with any work which he felt was not of the highest calibre. During the past few years he initiated studies on protein utilization and on rumen fermentation, using N^{15} and C^{14} as tracers.

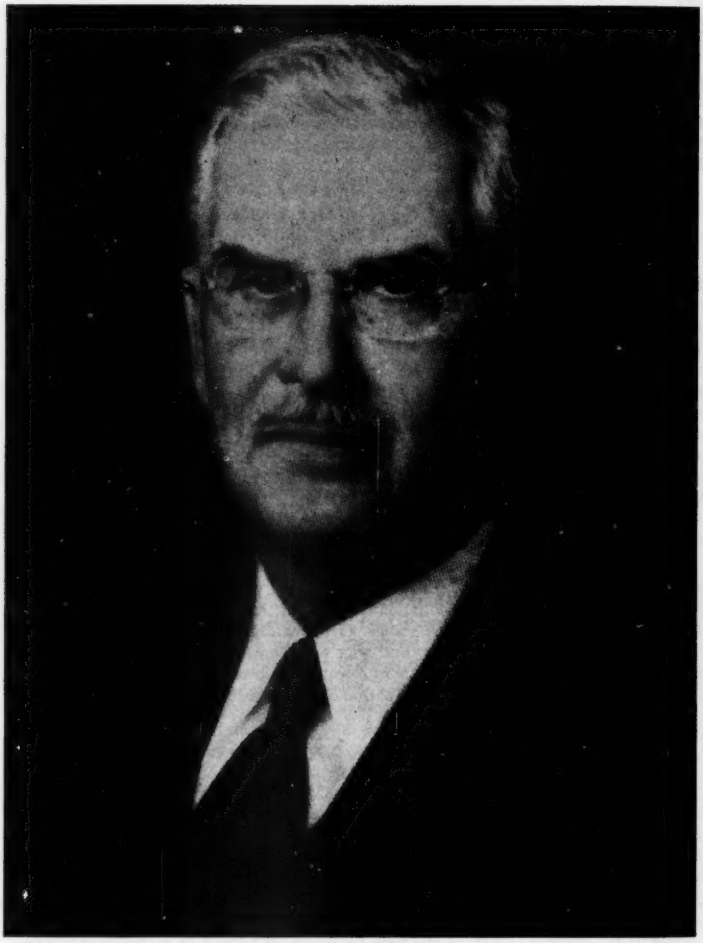
Dr. Watson was an active member of many local and international societies. He was a Fellow of the Chemical Institute of Canada, a member of the Agricultural Institute of Canada and of both Canadian and American Societies of Animal Production. For several years he served as secretary of the Canadian Council of the Society of Chemical Industry, and was well known to the Chemical Industry not only in North America but also in Great Britain and Europe. He was a vice president of the Vème Congrès International de Zootechnie held in Paris in 1949.

JOHN OSCAR WILLIAMS

Born at Venedocia, Ohio, March 10, 1885, Mr. Williams passed away September 24, 1950 at Falls Church, Virginia. He grew up on an Ohio farm and received a B.S. degree from Ohio State University in 1908. With the exception of four years' service as livestock specialist for Clemson College, his entire professional career was spent with the Bureau of Animal Industry, U.S.D.A. His first assignment was as animal husbandman in charge of the cooperative horse investigations at Fort Collins, Colorado from 1908 to 1914. From 1918 to 1936 Mr. Williams was stationed at Washington, much of the time in charge of horse and mule investigations. In 1936 he took over the supervision of the U. S. Morgan Horse Farm at Middlebury, Vermont where he was located until retirement from the service in 1945.

Mr. Williams was the author of many publications on the breeding, feeding, and management of horses. He was well qualified as a judge of horses of all kinds from ponies to the draft breeds and served at many of the leading shows in America. Some of these were Madison Square Garden, International Livestock Exposition, the Kentucky, Indiana and Ohio State Fairs.

Mr. Williams was a member of Alpha Gamma Rho fraternity; the National Press Club, Cosmos Club, and University Club, all of Washington, D. C. He was also a 32nd degree Mason. Surviving are his widow, a son and a daughter.



CARL WARREN GAY

Reproduction of a portrait presented to the Saddle and Sirloin Club in Chicago by the American Society of Animal Production on November 26, 1950. Carl Warren Gay was the Honor Guest of the Society during its annual meeting which was being held at that time.

CARL WARREN GAY

Honor Guest—1950

Carl Warren Gay was born in Waverly, New York, March 14, 1877. An only child, the son of a banker, he was graduated from Ithaca High School in 1895. He then enrolled in the college of veterinary medicine at Cornell University from which he graduated in 1899.

He was awarded a graduate fellowship at Cornell the next year and did his graduate work in bacteriology under the supervision of Dr. V. A. Moore. After completing his graduate experience at Cornell, Dr. Gay was employed by the New York State Board of Health in connection with a project planned by said Board of Health to control and to eradicate if possible Bovine Tuberculosis.

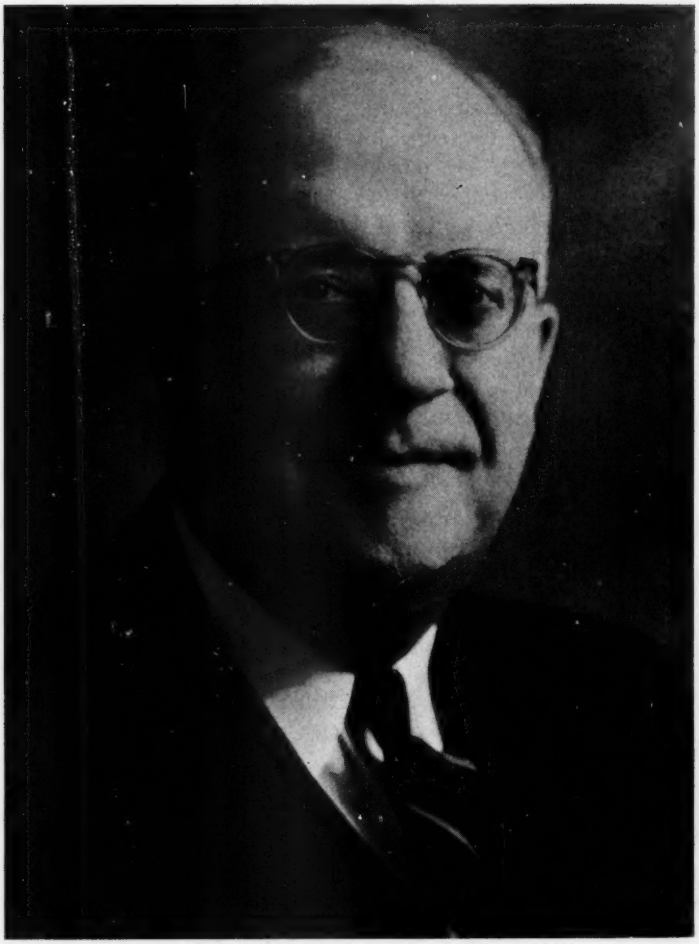
In September, 1901, Dr. Gay went to Iowa State College, Ames, Iowa as assistant professor in their college of veterinary medicine. While at Ames, Dr. Gay, in quest of additional training and experience in the livestock field, arranged to do part-time graduate work in animal husbandry. In 1903, he became a member of the animal husbandry department at Ames with the rank of assistant professor and with permission to continue his undergraduate studies.

In June, 1905 he received his undergraduate degree from the college of agriculture at Ames. In March, 1905 he came to Ohio State University for one term as assistant professor in animal husbandry, returning to Ames in June to graduate. In 1906, following graduation from Ames, he returned to Ohio State as associate professor in animal husbandry.

Dr. Gay remained on the staff at Ohio State until July, 1907, when he received a call to the University of Pennsylvania as professor of animal husbandry in their college of veterinary medicine. He remained there until 1916, when he went to the chairmanship of the department of animal husbandry at the University of Minnesota. He served the livestock interests in the Gopher State, until August, 1920, when he returned to Ohio State University as chairman of the animal husbandry division.

He served as animal husbandry chairman at Ohio State until 1940 when he asked to be relieved of the chairmanship responsibility. He continued his service at Ohio State in capacity of teacher of animal husbandry until he reached compulsory retirement age in 1947.

Upon the receptive minds of his students, lasting impressions have been made—impressions of a friendly man with a quizzical smile—a kindly man through whose veins courses the milk of human kindness, without which teachers and students suffer irreparable losses.



HAROLD HANSON MITCHELL

Harold Hanson Mitchell was awarded the fifth Morrison Award, which was presented to him during the 42nd Annual Meeting of the American Society of Animal Production in Chicago on November 25, 1950.

THE MORRISON AWARD

1950

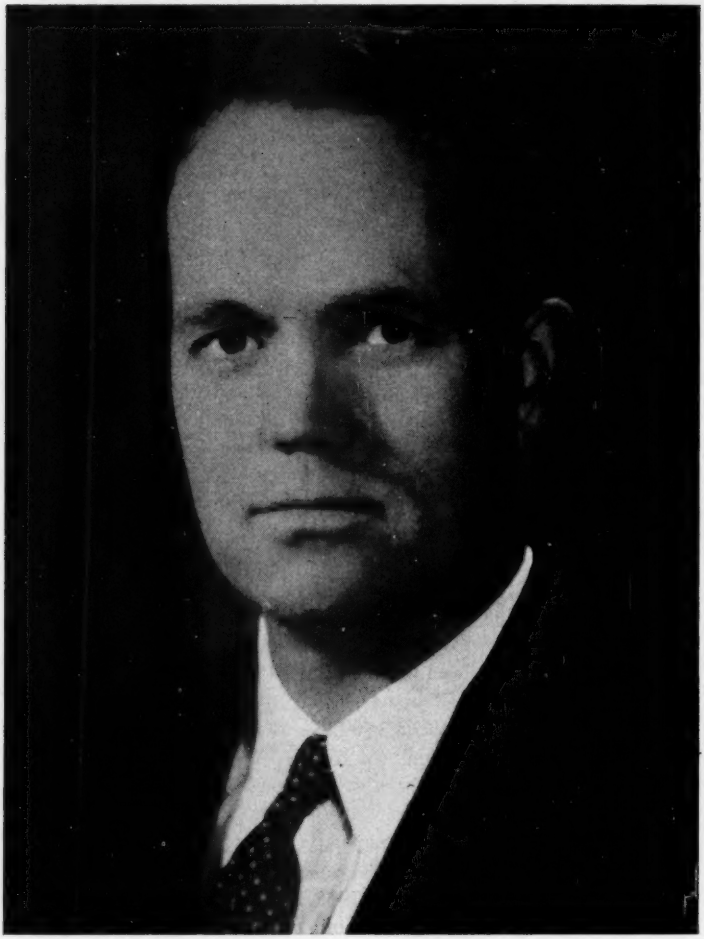
For his continuous and scholarly contributions to the knowledge of protein; its distribution in livestock feeds; its nutritive value in fresh, stored, and processed feeds and feed ingredients; and its needs by animals of various classes and for the purposes for which they are fed, the 1950 Morrison Award for meritorious scientific contributions in the field of animal husbandry goes to Dr. Harold Hanson Mitchell.

Of the 241 papers published by Dr. Mitchell since 1913, 82 have dealt specifically with problems of protein and 21 of these have been published during the period since 1945. One of the first to recognize the practical significance of quality of protein in livestock rations, his conception of biological value and of supplementary values of proteins in feeds, and his method for evaluating them, are perhaps his greatest contributions. His publications reflect a thoroughness of scientific approach and a searching appraisal of results which to the scientist are an inspiration, and to the layman instil confidence in the conclusions drawn. His ability as a fair and just critic has been recognized and appreciated by his contemporaries and has been directly and indirectly responsible for many noteworthy contributions to animal husbandry knowledge from other laboratories.

It is impossible to estimate the practical value of Dr. Mitchell's contributions to the feeders of livestock and to agriculture in general. There is no doubt, however, that the nation's ability to produce more meat, milk and eggs by healthier animals on more efficient and economical rations has been much enhanced by his researches.

Harold Hanson Mitchell was born January 22, 1886, in Evanston, Illinois. He received his A.B. in 1909, his M.Sc. in 1913, and his Ph.D. in 1915, all from the University of Illinois. As a biochemist and nutritionist he has been associated with the Illinois Agriculture Experiment Station since 1909; and he has been Professor of Animal Nutrition and Chief of the Division of Animal Nutrition since 1925. He was a member of the Committee on Animal Nutrition of the National Research Council from 1925 to 1945 and is at present a national collaborator of the U. S. Regional Soil, Plant and Nutritional Laboratories, Ithaca, N. Y. He received the Borden Award for the National Institute of Nutrition in 1945. Membership in various honorary and professional societies include: American Association for the Advancement of Science, American Chemical Society, American Society of Animal Production, American Society of Biological Chemists, American Institute of Nutrition, American Dietetic Association, Society of

Experimental Biology and Medicine, Sigma XI, Phi Lambda Upsilon, and Phi Kappa Phi.



JOHN KASPER LOOSLI

John Kasper Loosli was awarded the third American Feed Manufacturers' Association Award, which was presented to him during the 42nd Annual Meeting of the American Society of Animal Production in Chicago on November 25, 1950.

AMERICAN FEED MANUFACTURERS ASSOCIATION AWARD

1950

Dr. John Kasper Loosli was born at Clarkston, Utah, May 16, 1909. He received his Bachelor of Science Degree from Utah State College in 1931 and his Master of Science Degree from Colorado College in 1932. He completed the Doctor of Philosophy Degree in Animal Nutrition at Cornell University in 1938.

During the past 17 years he has held the following positions: Instructor of Agriculture, Utah Agricultural College, 1933–35; Assistant in Animal Nutrition, Cornell University, 1935–38; Bureau of Biological Survey, 1938–39; Assistant Professor of Animal Nutrition, Cornell University, 1939–43; Associate Professor of Animal Nutrition, Cornell University, 1943–45; Professor of Animal Nutrition, Cornell University, 1945 to date.

Dr. Loosli's name appeared on 21 scientific papers during the 1948–1949 period that was considered in making this award. He was senior author on five of these papers. Dr. Loosli's recent research has been concerned with vitamins A, E, and B_{12}. He has also participated in several studies concerning rumen synthesis. Other studies have dealt with the mineral requirements of domestic animals, especially those for cobalt and sulphur.

While much of Dr. Loosli's research and many of his publications are of fundamental importance to the field of nutrition, he also possesses the happy faculty of being able to apply research findings to farm problems and to express himself in a manner that can be easily understood by the layman. His work with graduate students at Cornell University has been outstanding. His cooperative attitude and skillful guidance has endeared him to many young men who will soon take the lead in nutritional work throughout the country. Dr. Loosli's high moral standards, his friendliness, and his unassuming manner, as well as his high professional attainments have won the respect of his fellow workers in Animal Nutrition. The fact that Dr. Loosli has reached such a high level of achievement at such an early period in his life gives promise of much outstanding work to come.

CHAIRMEN OF COMMITTEES, PROGRAM CHAIRMEN AND SPECIAL ASSIGNMENTS FOR 1951

Standing Committees

Committee on Investigation.....C. E. Terrill
Committee on Instruction.....E. H. Hughes
Committee on Extension.....Graydon Blank
Committee on Membership.....H. M. Briggs

Special Committees

Pasture and Range Investigation.....G. O. Mott
Committee on Information.....R. C. Pollock
Committee on Necrology.....E. H. Hostetler
Nominating Committee.....L. A. Weaver
Committee on Morrison Award.....H. M. Briggs
Committee on American Feed Manufacturers' Award.....L. L. Madsen

Program Chairmen

Breeding and Genetics Section.....L. N. Hazel
Extension Section.....Geo. R. Johnson
Instruction Section.....E. H. Hughes
Meats Section.....A. L. Anderson
Nutrition Section.....Wise Burroughs
Pasture, Forage and Range Section.....L. E. Harris
Physiology Section.....H. H. Cole

Special Assignments

Importation of Livestock.....H. R. Guilbert
Inter-Society Association Council.....E. A. Livesay
Historian.....C. W. Gay
American Association for Advancement of Science Council.....F. F. McKenzie
Division of Biology and Agriculture, N.R.C.....H. W. Marston
European Animal Husbandry Association.....R. W. Phillips
Ralston Purina Award Committee.....W. M. Beeson

NEWS AND NOTES

Applications for the Ralston-Purina research fellowship should be on file by March 1. Application blanks may be obtained from the Ralston-Purina Research Awards Committee in care of Mr. J. D. Sykes, Ralston-Purina Co., St. Louis, Missouri. The fellowships are for $1440 annually and awards will be made in the fields of nutrition and physiology research as applied to dairy, poultry and animal husbandry, and in research in transmissible diseases of livestock and poultry. Individuals qualified for graduate study in any Land-Grant Agriculture College and approved Veterinary Colleges are eligible.

A graduate fellowship of not less than $500 at Cornell University for qualified students majoring in livestock feeding has been established by F. B. Morrison to promote research in livestock feeding and nutrition. The award, to be known as the Morrison Fellowship in Livestock Feeding, is open to either foreign or native students with major consideration given to their qualifications for graduate study and need.

R. S. Glasscock was official judge of Brahman cattle at their show in Bogota, Colombia, Dec. 2–13. He also judged all breeds of beef cattle at the show in Sancti Spiritus, Cuba, December 14–21.

An Inter-American meeting on Livestock Production was held at Turrialba, Costa Rica, from 9 to 20 October, 1950, under the joint auspices of the Food and Agriculture Organization of the United Nations and the Inter-American Institute of Agricultural Sciences. Delegates were present from 11 countries and observers from 5 organizations. The meeting dealt with problems in the fields of animal nutrition, range and pasture improvement, livestock management, climatic physiology, animal breeding and veterinary medicine. Ralph W. Phillips of FAO, B. T. Simms of the U. S. Bureau of Animal Industry, Douglas H. K. Lee of Johns Hopkins University, and A. O. Rhoad of the Institute, served as technical secretaries.

Eleven men who have rendered 25 years or more of outstanding service in the meats field were signally honored by the National Live Stock and Meat Board at its 1950 annual meeting. Citations sketching their achievements were made by Jay Taylor, Amarillo, Tex., Vice chairman of the Board. The men honored were Phil Anderson, University of Minnesota; Sleeter Bull, University of Illinois; J. B. Francioni, Louisiana State University; Wm. J. Loeffel, University of Nebraska; D. L. Mackintosh, Kansas State College; A. W. Oliver, Oregon State College; Roy Snyder, Texas A. and M. College; E. J. Wilford, University of Kentucky; P. T. Ziegler, Pennsylvania State College; O. G. Hankins and Kenneth F. Warner, U. S. Department of Agriculture.

Lorin E. Harris, formerly associate professor of animal husbandry has been appointed Chairman of the Institute of Nutrition and professor of animal husbandry at the Utah State Agricultural College. In this new position he will correlate the nutritional research in the various departments of the College.

American scientists and technologists are being asked to participate in registrations of scientific personnel to provide urgently needed information on the country's supply of technological personnel in the many scientific fields—information needed for both immediate and long-range mobilization planning. This is an operation of the National Scientific Register, sponsored by the National Security Resources Board, and administered by the Office of Education, Federal Security Agency. The registration includes qualified scientists in the natural and physical sciences, and is being aided by the principal professional and scientific societies.

James O. Grandstaff, Superintendent of the Southwestern Range and Sheep Breeding Laboratory, is spending three months (January through March) in Libya for the Food and Agriculture Organization of the United Nations, making a survey of the sheep and wool industry and advising on methods of improving the industry.

The Food and Agriculture Organization of the United Nations is transferring its headquarters from Washington, D. C. to Viale delle Terme di Caracalla, Rome, Italy. Members of the American Society of Animal Production who will move to Italy with the Organization include Ralph W. Phillips, Deputy Director of the Agriculture Division, A. T. Semple, Range Management Specialist and N. R. Joshi, Animal Breeding Specialist in that Division. The transfer of Hq. will be completed in April 1951.

VOL
1

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should contain a clear statement of the purpose of the investigation and its relation to other work in the same field, but long reviews should be avoided. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results, or their relation to some problem of the livestock industry. Each paper should end with a brief summary outlining the points proved in the investigation and their application, or their relation to a livestock problem.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. A maximum of eight pages per paper will be published without charge. Over-run in excess of this amount will be charged to the author or his institution at current publication costs. This restriction does not apply to invited review papers. The cost of making engravings, in excess of one page per paper and of printing tables in excess of two pages per paper, will also be charged to the author or his institution.

6. Authors will receive galley proof of articles, together with a reprint order form and a form for supplying an abstract for "Biological Abstracts." Corrected proof, reprint order and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue. Institutional orders for reprints should be made out to the American Society of Animal Production.

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin.

12. All correspondence concerning subscriptions to the JOURNAL and other business matters should be addressed to the Business Manager of the JOURNAL, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y.